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Studies on Phytophthora Seed Piece Rot of Sugarcane and the Principal Causal Organism Phytophthora Megasperma, Drechs.

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STUDIES ON PHYTOPHTHORA SEED PIECE ROT OF SUGARCANE AND THE PRINCIPAL CAUSAL ORGANISM P. MEGASPERMA DRECHS.

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ABSTRACT

A study was made of the Phytophthora seed piece rot of sugarcane in Louisiana. The principal causal organism of this disease has been known for many years as a "sterile" Phytophthora isolate, due to the absence of fruiting structures, while a lesser pathogenic isolate had been identified as P. erythroseptica Pethyb.

In the present study, it was found that the "sterile" isolate will produce fruiting structures, when the fungus is grown on sterilized oat grains. After these grains were placed in tap water, many sporangia and chlamydospores were also observed. A complete description of this isolate has been given.

After comparative studies with several type cultures of seemingly related species of Phytophthora, in respect to morphology, growth-temperature relations and pathogenicity, the principal causal organism of sugarcane seed piece rot has been identified as P. megasperma Drechsler. The isolate was found to have an optimum growth temperature of 20°-22° C., with a range from 6°-32° C.

In basic infection studies, it was found that the initial infection of the seed pieces takes place at the root primordia and buds of each node. After having been planted 9 weeks in sterilized, artificially infested soil, the variety C.P. 36-13 showed 67.9 per cent of the root primordia and 80.0 per cent of the buds infected, this being

somewhat less for the variety N.Co. 310. Since it was also noticed, that none of the still healthy root primordia and buds of the seed pieces planted in infested soil had germinated, it is suggested that the fungus may be producing a toxin, which inhibits the germination process.

In order to study the rate of spread of Phytophthora inside the cane, the internodes of 2-eyed seed pieces of the varieties C.P. 36-13 and N.Co. 310 were inoculated with the isolate. It was observed that the fungus reached the nodal regions 20 days after inoculation, while after 50 days the internode had almost entirely deteriorated.

In a comparative susceptibility experiment, in which the varieties P.O.J. 213, Co. 290 and C.P. 36-13 were tested in regard to red rot and Phytophthora rot, it was found that C.P. 36-13 was most susceptible to Phytophthora rot, while the other two varieties were about equal in their reaction to the disease.

An extensive varietal field test was carried out during the winter and spring seasons of 1957-1958 and 1958-1959, in which many past and present commercial sugarcane varieties and some unreleased test field varieties were studied in regard to their susceptibility to Phytophthora seed piece rot. From the data collected during this two year testing program, it is concluded that the varieties C.P. 28-19, C.P. 34-120, C.P. 36-13, C.P. 44-154, C.P. 53-1, C.P. 53-15, C.P. 53-22 and C.P. 55-30 are susceptible to the seed piece rot,

while the following varieties are considered resistant: Co. 281, N.Co. 310, C.P. 29-116, C.P. 43-47, C.P. 44-101, C.P. 47-193, C.P. 48-103 and C.P. 51-21.

A survey made in the sugar belt during the spring of 1958, revealed the fact that higher percentages of Phytophthora cultures were obtained from seed pieces planted from late September until November than from those planted earlier.

INTRODUCTION

The rotting and deterioration of sugarcane seed pieces or cuttings is a problem present in almost every sugarcane growing country in the world. In the tropical areas, pineapple disease caused by Ceratostomella paradoxa (de Seynes) Dade is considered the most important seed piece rot, while in the subtropical areas red rot caused by Physalospora tucumanensis Speg. is the one causing most damage in this respect.

Since in Louisiana, the plant cane lies dormant in the soil for about six months after it has been planted in September and passes through a period of unfavorable climatic conditions during this time, the vitality of the cane is easily lowered and the seed pieces become more susceptible to the attack of certain soil microorganisms. Sometimes this winter period has been experienced to be unusually wet accompanied by extra low temperatures. This condition was observed during the winter and early spring of 1947 and 1948, and it was found that the variety C.P. 36-13 failed to germinate in certain areas of the sugarcane belt. Since this variety was considered as being highly resistant to red rot it was hard to believe that this could be the cause of the failure. It was also considered to be resistant to mosaic and moderately so to root rot (12).

In the early spring of 1948, a survey was made of the fungi associated with the stand failures in plant cane, and isolations

revealed several strains of Phytophthora to be responsible for the malady. Chilton and Steib (18) gave the first account of this Phytophthora rot in 1948, which report was followed by more detailed publications a few years later (79, 80). When seed pieces of the variety C.P. 36-13 and Co. 290 were re-inoculated with cultures of Phytophthora, symptoms similar to those seen in the field were obtained.

In the early stage of infection, the disease could also be distinguished from red rot by the internal symptoms of the diseased seed pieces. Red rot is characterized by a deep red discoloration of the internal tissue and the presence of typical transverse white blotches in the internodal region, while Phytophthora rot shows a typical orange-pink discoloration of the tissues, without the presence of any white spots.

Upon examination of the Phytophthora strains isolated in 1948, one was identified as P. erythroseptica Pethyb. Of the remaining, one was found to resemble Pythium in its morphological characteristics, while the other was never identified due to the complete absence of any fruiting structures, and which was therefore tentatively called the "sterile mycelium" isolate. This last isolate, however, was found to be the most pathogenic one in seed piece inoculation experiments.

Sanchez-Navarrete (71) in 1950, made a study of the environmental factors relative to Phytophthora rot of seed pieces and concluded that the yield of sugarcane in tons per acre was directly related to the

temperature and the amount of rainfall during the preceding winter and spring seasons. Lower average temperatures and excessive amounts of precipitation usually resulted in lower yields of sugarcane. This investigator also made attempts to induce fruiting in the "sterile," most pathogenic Phytophthora isolate, but only chlamydospores were observed.

In 1955, Singh (75) continued these attempts through the use of different culture media, fleshy fruit, soil leachate and other means, but was still unable to obtain any fruiting structures in this isolate with the exception of few sporangia and oospores observed on sugarcane roots. He also made extensive surveys in the sugarcane belt during the spring seasons of 1953, 1954 and 1955 in order to determine to what extent Phytophthora was important in causing plant cane stand failures.

Since it has been definitely established in previous investigations, that the "sterile" Phytophthora isolate is the most pathogenic one in respect to Phytophthora seed piece rot, all emphasis was placed entirely on that particular isolate in this study.

The present investigation was undertaken, (1) to try to induce fruiting in the "sterile" strain and to identify this pathogenic Phytophthora isolate, (2) to make comparative studies between this isolate and other species of Phytophthora in regard to morphology, growth-temperature relations and pathogenicity, (3) to study the basic

method of seed piece infection and the rate of spread of the pathogen inside the seed pieces, and (4) to determine the relative susceptibility of several past and present commercial sugarcane varieties as well as some yet unreleased experimental test field varieties to the disease under field conditions.

HISTORICAL REVIEW

Of all the recognized sugarcane diseases today, several are known which actually involve a rotting of the seed pieces, some being of major importance. Different terms used for the word seed piece in other sugarcane growing countries are "cuttings" in Australia, Hawaii, Formosa and the Philippines, "setts" in India, South Africa and Mauritius, (also "bouture"),"stekken" and "bibit" in Java, and "semillas" in Puerto Rico and other spanish speaking countries.

According to Rands and Abbott (68), a rot of "mattressed" seed cane, also called "cane rot," was vaguely described in the 1840's and 50's in Louisiana, which seemed to be associated with the degeneration or falling off in yield of the long-cultivated Creole and Otaheite cane varieties.

Probably the earliest report of a definite seed piece rot of sugarcane came from Java in 1893, when Went (94) reported the "ananas ziekte" or pineapple disease, which caused considerable damage at that time. Went described the causal organism as Thielaviopsis ethaceticus and used the name pineapple disease because of the odor given off by the sugarcane seed pieces in the early stages of rotting, resembling that of fresh pineapples.

Since de Seynes (26) had studied and identified this fungus earlier from rotting pineapple fruits, the name was changed to T.

paradoxa. It was not until 1928, that the perfect stage was described by Dade (24) from the Gold Coast and the fungus was renamed Ceratostomella paradoxa. This name has very recently been changed by Moreau (61) to Ceratocystis paradoxa (Dade) Moreau.

This seed piece rot is characterized by a blackening of the central cylinder of the stalk and the release of an odor resembling that of fresh pineapples. These characteristics in addition to the fact that this disease is more severe at higher temperatures, distinguish it from Phytophthora seed piece rot. The disease is present in all sugarcane producing countries throughout the world and is the principal seed piece rot in Hawaii today. Ocfemia (62) reported from the Philippines, that in 1925 shoots failed to emerge from 95 per cent of the cuttings planted in one of the areas, owing to this disease. Pineapple disease was first found in Louisiana by Edgerton (30) in 1910, but the disease has been of infrequent occurrence and of little economic importance in the state. Cook (20) reported from Puerto Rico in 1932, that C. paradoxa was the dominant factor in poor germination of the seed cane and that destruction of the seed pieces was greatest in wet, poorly drained soils.

The fungus enters the seed pieces from the soil through the cut ends and grows rapidly in the central part of the cane stalk, eventually destroying the seed piece entirely. The severity of the disease is increased by conditions unfavorable for germination of the buds, like

poor drainage and little aeration of the soil.

In respect to the control of pineapple disease, Went (94) already recommended coating of the cut ends of the seed pieces with some tar mixed with arrack. This subject was later investigated by Kamerling (47), who compared the treatment with Bordeaux mixture and the use of tar alone. Evans and Wiehe (35) in Mauritius, McMartin (58) in South Africa and more recently Wismer (96) in Hawaii have published extensive articles on the control of pineapple disease by the use of organic mercurial fungicides. The first two investigators reported that Aretan gave the best protection of the setts under both moist and dry conditions, while the latter recommended phenyl mercuric acetate (PMA) to give the most effective control of the disease at a concentration of 1 quart to 100 gallons of water, for dipping or spraying of the cuttings.

Pineapple disease was quickly followed by the occurrence of another seed piece rot described from Java by Went (95) in 1893, which disease he called "rood snot" at that time. Went described accurately and in detail the symptoms of the disease, proved the parasitism of the causal organism and named it Colletotrichum falcatum. The perfect stage of the red rot fungus was reported by Carvajal and Edgerton (16) from Louisiana in 1943. After comparison with type material, it seemed identical with a fungus described by Spegazinni (76) in Argentina as Physalospora tucumanensis, which name is used today

for the red rot fungus. Recently, however, von Arx and Muller (87) changed the name to Glomerella tucumanensis (Speg.) v. Arx et Muller, on account of the absence of paraphyses in the latter genus.

This seed piece rot is characterized by the reddening of the internodal tissues and the presence of white blotches, which together result in a typical mottling effect. This symptom can definitely be differentiated in the early stage of seed piece infection from the typical orange pink discoloration of cane rotted with Phytophthora.

Red rot has been considered as one of the chief causes of stand failures all over the world. It was reported from Louisiana for the first time in 1908 by Edgerton (29) and has since then caused severe losses to the Louisiana sugar industry. In 1920, the same investigator reported that inoculation of seed pieces of the varieties Louisiana Purple and D. 74 with the fungus, decreased germination of the eyes by nearly 50 per cent. By 1927, the variety P.O.J. 213 had become one of the leading commercial varieties in the state as a replacement of the old noble canes, Louisiana Purple and Louisiana Striped, and at that time, was considered resistant to red rot. In the spring of 1930 when the variety occupied about 35 per cent of the sugarcane acreage in Louisiana, it failed to germinate in many areas in the state and examination of the seed pieces revealed extensive injury by the red rot fungus (3).

Abbott (3) considered various possibilities as being the cause

of this failure, but placed most emphasis upon the possible development of a new strain of the red rot fungus, being more virulent to P.O.J. 213 than to those varieties present in the state before its introduction. An extensive survey was made of the red rot flora in the Southern United States, which revealed the first evidence of the existence of two morphological types of C. falcatum, which were termed light and dark races respectively.

Forbes (36, 37) in 1942, investigated the deterioration of Co. 290 seed cane in Louisiana, and found that red rot began to play an important role in the poor germination of seed pieces of that variety. The variety Co. 281, however, was found to be much more resistant to the disease.

Cytospora rot of sugarcane is commonly referred to as a sheath rot, but it has been found to affect the seed pieces and stubble rhizomes as well. The fungus was first described by Butler (14) from India in 1906 as Cytospora sacchari Butl. In the Western Hemisphere, the organism was first reported by Johnston and Stevenson (45) from Puerto Rico in 1917. The disease was first found in Louisiana on the variety Co. 281 by Abbott (1) in 1930, when the industry was shifting its varieties to the newer hybrids. According to this investigator, Cytospora rot had not been considered a disease of seed cane until 1936, when stands of C.P. 28-19 were poor over considerable areas in Louisiana. The deteriorated seed pieces dug from the gaps were in

most cases found to be spiny with fruiting bodies of the fungus. He also reported that the varieties C.P. 807 and C.P. 29-320 were infected with Cytospora, while the varieties Co. 290, P.O.J. 36, P.O.J. 213 and P.O.J. 234 were only slightly affected. Similar conditions were observed during the spring of 1937, while the disease was found to be most severe on poorly drained areas that had been excessively wet for considerable periods during the previous winter.

Chilton (17) made an extensive study of this disease during 1935-1936 and confirmed previous reports, that C.P. 28-19 and C.P. 29-320 were the most susceptible varieties to Cytospora rot. He reported also, that wherever germination had failed to take place, pycnidia of the fungus could be found upon the seed pieces. Cane, affected by C. sacchari, has a rind which turns dull brown to black in color, the surface of which is rough due to the spiny fruiting bodies in the epidermal tissue, while the internal tissue becomes a dirty gray, later turning darker.

Another seed piece rot, known as black rot, was originally found and described by Butler (14) from Bihar, India in 1903. He identified the causal agent as Sphaeronema adiposum, which name was later changed by Sartoris (72) to Ceratostomella adiposum (Butl.) Sartoris, on account of the typical long-beaked perithecia which Butler had mistaken for pycnidia. The name black rot could be confused with the term pineapple disease, since Went (94) and Krüger (48) also called the

latter disease "zwart rot" and "Schwarzfaule" respectively.

Sartoris (72) reported in 1927 that black rot caused considerable damage to the seed cane of the varieties P.O.J. 36 and P.O.J. 213 and that in the fall of 1926, just prior to cane harvesting, the stands of these two varieties were reduced to about 20 and 30 per cent of normal respectively. He also mentioned that ideal conditions for the development of the disease were frequent rains with intermittent periods of warm weather, combined with loosely packed soil allowing large air pockets around the seed pieces. The mycelium did not seem to invade the seed pieces, however, but turned the internal tissue a dark purple. In advanced stages the seed pieces turned nearly black throughout, showed a watery texture and emitted an odor similar to that of the pineapple disease.

Black rot has now been reported in the Western Hemisphere from the United States, Brazil, Peru and the Dominican Republic and from the Eastern Hemisphere from Australia (New South Wales), Java, India and Formosa (56).

Fusarium moniliforme (Sheld.) Snyd. et Hans. has also been isolated from the internal tissues of deteriorating seed pieces. Edgerton and Moreland (32) described the disease from Louisiana and also two distinct types of Fusaria associated with it, a white and a purplish species. The purple Fusarium was found to be a large-spored form, producing deep purple fruiting pustules on the outside of the seed cane

during late winter, while the white form had spores much smaller than those of the purple Fusarium, and was found present in almost every stalk of discolored seed cane.

They reported also that Co. 290, which was of commercial value at that time, was most susceptible to Fusarial rot, especially since it affected seed pieces planted in low, wet areas. Experimental evidence indicated that the purple Fusarium was of minor importance in the deterioration of seed cane, while inoculation of the seed pieces with the white Fusarium consistently resulted in lower bud germination than in the check, which indicated that this fungus could be of some importance in causing a decrease in stand.

Abbott (2) reported a reduction in germination due to Fusarium in P.O.J. 213, where the inoculated plots showed 5.8 per cent germination as compared with 9.9 per cent for the check plots.

McMartin (57) reported a sett-rot of sugarcane from Natal and stated that a number of different organisms, the names of which were not mentioned, were associated with the disease. He found Co. 301 to be one of the most susceptible varieties to sett-rot and was also able to control the disease through the use of organic mercurial compounds applied in the furrow.

A report from Australia by Hughes and Christie (42) in 1950 stated that treatments of sugarcane setts in the Budehin district with a mercuric dip prior to planting had resulted in a tremendous stand

increase and that the treatment had become a regular practice in that part of the Australian sugarcane area.

Johnston (44) reported several fungi from Puerto Rico which he isolated from seed pieces removed from the soil: sclerotia and mycelium of Sclerotium rolfsii Sacc., fructifications of Diplodia theobromae (Pat.) Nowell and Cytospora sacchari Butl., and also Melanconium sacchari Mass. He stated, however, that there was no proof that any one of these organisms might not have gained entrance to the seed pieces before planting.

Bitancourt (10) listed the same fungi from seed pieces in Brazil, but reported them as being of minor importance.

Edgerton (30) also reported the presence of rind disease, caused by Melanconium sacchari, from seed cane in Louisiana and stated that the interior of the seed pieces was variously colored, ranging from red and yellow to brown and even green. The tissue was reported to dry out rapidly so that the whole cane shrinks and becomes light in weight, while little or no germination was observed.

Abbott (2) in 1932, found that M. sacchari reduced the amount of germination of the variety C.P. 807 from 32.9 per cent in the check to 19.4 per cent in the inoculated seed pieces, while germination in the variety P.O.J. 213 was reduced from 14.5 to 9.4 per cent.

Marasmius plicatus Wakker, now commonly known as M. stenophyllus Mont., has also frequently been seen on seed cane in

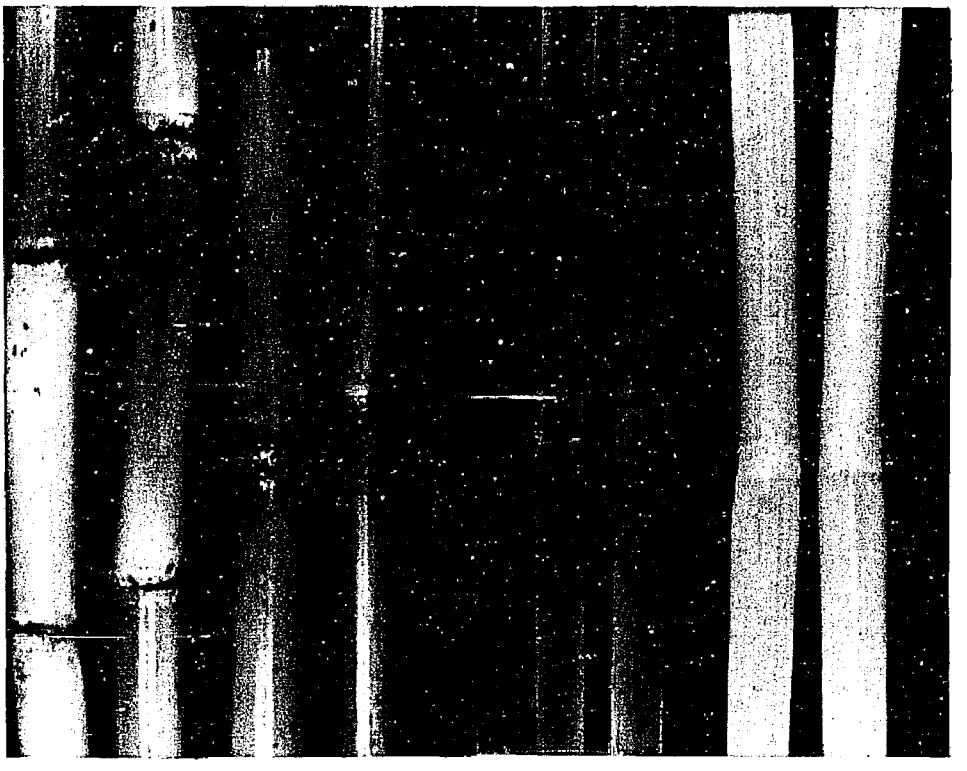
Louisiana when diseased stalks were used for planting (32). It was reported at that time that the fungus did not penetrate the rind tissue from the leaf sheaths, but mycelium was found entering the stalk through borer holes or other injuries of the seed cane.

Phytophthora rot of sugarcane seed pieces, the disease under study in the present investigation, has been recorded only from Louisiana up to the present time. The disease is characterized by a water soaked appearance of the infected internal tissues followed by a typical orange-pink discoloration (Plate I). In the more advanced stages of infection, the tissue turns reddish and purplish brown and has often a distinct ether-like odor.

In the spring of 1947 and 1948 it was evident that the variety C.P. 36-13 was showing stand failures in certain parts of the Louisiana sugarcane belt. Steib and Chilton (79, 80) made a survey of the areas and isolations were made from the ungerminated seed cane. They reported that of 2,017 nodes and internodes plated, 51.5 per cent gave Phytophthora isolates, which were divided into 3 distinct cultural types. These three isolates were tentatively called "strains" Nos. 1, 2 and 3.

Strain No. 1, characterized by the production of oogonia and antheridia on oatmeal agar and abundant sporangial production, was identified as P. erythroseptica Pethyb. (78). Strain No. 2, characterized by the absence of sexual structures and the production of few sporangia, was referred to as the "sterile-mycelium" type. Strain No. 3,

PLATE I



External and internal discoloration of seed pieces of the variety C.P. 44-154 infected with Phytophthora, as contrasted with the natural color of healthy cane.

characterized by the production of only paragynous antheridia, and sporangia as in strain No. 1, was referred to as a Pythium-like Phytophthora. It was also noted at that time that strain No. 2, with "sterile" mycelium, was most pathogenic of the three isolates, whereas strain No. 3 was least pathogenic. Singh (75), who worked on this disease recently, was able to isolate only strains Nos. 1 and 2, which he designated as Isolate No. 1 and Isolate No. 2.

Sanchez-Navarrete (71) was the first to make attempts to induce the formation of fruiting structures in this "sterile" Phytophthora isolate, which had obviously been the critical point preventing it from identification. He mated this isolate with the other strains mentioned from sugarcane and also with P. infestans (Mont.) de Bary from potato, P. cinnamomi Rands from Camellia and a Phytophthora-like fungus from sweet potato, but no fruiting structures, whether sexual or asexual, were ever observed at the line of junction between the Phytophthora's. When this "sterile" strain was placed in Petri's mineral solution and M/100 potassium nitrate solution, only chlamydospore-like bodies were found in abundance.

Singh (75) made further attempts to induce fruiting in the "sterile" isolate, by using different media, fleshy fruit, soil leachate, sugarcane stalks and roots, the effect of light and paired cultures. Of all the materials used, only the inoculation of sugarcane roots resulted in a limited production of sporangia and oogonia. The use of different

media and soil leachate, with or without Petri's solution, seemed to be very favorable for chlamydospore production only. Regardless of these findings the fungus was not identified at that time.

MATERIALS AND METHODS

Steib and Chilton (78) isolated three morphologically different Phytophthora isolates from diseased sugarcane seed pieces, which were designated as Strains 1, 2 and 3. Since the "sterile" Phytophthora isolate (Strain No. 2) was considered to be the most pathogenic by these and later investigators (71, 75), this isolate was used in the present study of this disease.

The work consisted of laboratory studies, in order to try to identify this previously undescribed isolate; greenhouse experiments to study the initial infection of the seed pieces by the pathogen and the spread of the fungus internally; and field tests to determine the susceptibility of many sugarcane varieties to this seed piece pathogen.

Laboratory Studies

Sanchez (71) and Singh (75) had previously tried to induce fruiting in this "sterile" Phytophthora isolate in order to obtain sporangia and oospores, but all attempts were unsuccessful. The latter investigator did find a few fruiting bodies, however, when roots from sugarcane seed pieces were allowed to grow in flasks with sterile water containing the fungal isolate.

Since these previous investigators never reported the examination of oats with the fungus, which was the inoculum also used at that time

for soil infestation and seed piece inoculation studies, this medium was thought of as a possibility for the fungus to fruit upon.

Therefore, in the present study, the fungus was grown on sterilized oats in Erhlenmyer flasks, which inoculum was intended for use in the field tests, to be planted with the seed pieces in the furrow. These flasks were stored in a constant temperature room at 70° F., and the oats were examined at different intervals for the presence of any fruiting of the fungus. Oat grains with the fungus were also placed in Petri dishes with tap water to test for sporangial production. A somewhat similar method was used recently by Lowy (55) in the study of aquatic Phycomycetes. This technique was later improved by the author, by placing the oat grains on wooden rods held with cotton plugs in Erhlenmyer flasks with water. This improvement allowed the fungus to grow out more in all directions.

For comparative studies, this "sterile" sugarcane isolate was compared with other species of Phytophthora. P. cinnamomi from avocado, P. erythroseptica from potato, P. drechsleri and P. megasperma from unknown hosts were obtained from the American Type Culture Collection (A.T.C.C.) in Washington, while a culture of P. cryptogea from alfalfa was kindly supplied by Dr. D. C. Erwin, Citrus Experiment Station, Riverside, California.

A temperature study was set up in which all the above mentioned species of Phytophthora and the sugarcane isolate were compared at

5°, 10°, 15°, 20°, 25°, 27°, 30° and 35° C. The isolates were grown in similar sized Petri dishes with 20 cc. potato dextrose agar, by placing a 12 mm plug of each culture in the center of the dish. The plates were inoculated at the required temperatures and examined 6, 10 and 30 days later to obtain measurements of the colonies.

The Phytophthora species, which morphologically resembled the sugarcane isolate most, were tested on five additional culture media at temperatures of 10°, 20° and 32° C., to compare them again for any differences in growth. The isolates were used from cultures growing on potato dextrose agar and the initial inoculum was reduced to 10 mm. The following media were used in the temperature studies (27, 54, 69):

Oatmeal Agar

Oatmeal	65	gr.
Bacto agar	17	gr.

Cornmeal Agar

Cornmeal	20	gr.
Bacto agar	17	gr.

Potato Dextrose Agar

Potatoes (peeled)	200	gr.
Dextrose	20	gr.
Bacto agar	17	gr.

Lima Bean Agar

Lima beans (ground)	100	gr.
Bacto agar	17	gr.

Czapek Agar

Sodium nitrate	2	gr.
Potassium phosphate (dibasic)	1	gr.
Magnesium sulphate	0.5	gr.
Potassium chloride	0.5	gr.
Ferrous sulphate	0.01	gr.

Fries Agar

Ammonium tartrate	5	gr.
Ammonium nitrate	1	gr.
Potassium phosphate (dibasic)	1	gr.
Magnesium sulphate	0.5	gr.
Sodium chloride	0.1	gr.
Calcium chloride	0.13	gr.
Yeast extract	0.01	gr.
Bacto agar	15	gr.

All the above media were made up in 1000 cc. distilled water. The plates were examined 6 days after incubation to obtain diameter measurements of the colonies. Both temperature experiments were run with 4 replications for each treatment and the experiment was repeated once.

P. megasperma and the sugarcane Phytophthora isolate were also compared in respect to their pathogenicity on apples, eggplants and oranges, the standard fruit used by Tucker (85) and Wager (89) in their comparative studies of Phytophthora species. These fruit were surface sterilized by dipping into a 1:1000 solution of mercuric chloride and were then placed in moist chambers, while the inoculations were made by placing with a sterile needle a 12 mm disk of agar with mycelium in a small, shallow wound cut into the surface of these fruit. Check fruit were wounded only. The moist chambers were then incubated at 21° C. and the fruit examined after 7 days.

These same two Phytophthora isolates were also tested with respect to their pathogenicity to sugarcane seed pieces. Since P. erythroseptica had been described from seed pieces before, also this species was used in the comparative test. All three Phytophthora cultures were inoculated into a total of ten 2-eyed seed pieces of the varieties Co. 290 and C.P. 34-120, by placing about 5 oat grains with the fungus in a hole cut into the internode of each seed piece. The seed pieces were then planted in large crocks with sterilized

soil, which in turn were placed in a constant temperature room of 70° F. (21° C.). The canes were dug and split 15 days after planting, to be examined for spread of the fungi.

Greenhouse Experiments

In order to study the initial infection of sugarcane seed pieces by Phytophthora, an experiment was set up in which 2-eyed seed pieces were planted in crocks filled with sterilized soil, to which the fungus was added. The inoculum was prepared by growing the fungus on sterilized oats. The seed pieces of the varieties C.P. 36-13 and N.Co. 310 tested were positively free of borer holes, in order to eliminate possible entrance of the fungus through such avenues.

A total of 150 single eyes, including 50 for the check, were planted in the experiment. The cane pieces were examined 2, 3, 5, 7 and 9 weeks after planting, by digging up 15 2-eyed seed pieces at one time, including 5 for the check.

In order to test the rate of spread of the pathogen in sugarcane seed pieces, a similar experiment was set up as described above, with the exception that the cane pieces were inoculated with the fungus, by cutting a hole in each internode and placing 5 oat grains with the fungus in each hole. A similar number of 2-eyed seed pieces was planted as in the previous experiment, and the canes were dug, split and examined 10, 15, 20, 30 and 50 days after planting.

Both experiments were set up outside the greenhouse during February and March, when the temperature varied from 44°-58° F. (6°-14° C.), while the crocks were kept well watered at all times.

Because of the fact that in Louisiana P.O.J. 213 suddenly showed stand failures in the 1930's, Co. 290 in the early 1940's, and C.P. 36-13 in the late 1940's, it was thought to be of some importance to try to determine the cause of these failures in respect to Red rot and Phytophthora rot. In order to do so, an experiment was set up outside the greenhouse during the winter months of 1958-1959, in which the aforementioned varieties were tested. A total of 12 boxes were assembled from 24 sweet potato crates, which were lined with cardboard and then filled with soil previously sterilized in the greenhouse sterilizer (Plate II). Each box contained 8 flats of soil and 4 boxes were used for each of the three varieties. Each variety was planted in 4 treatments with 10 replications as follows: (1) check with uninoculated seed pieces planted in uninfested soil, (2) the seed pieces were inoculated with the red rot fungus, by cutting a hole in the center of each stalk and placing about 2 cc. of a conidial suspension of Physalospora tucumanensis in each hole, (3) the seed pieces were planted in soil previously infested with the sugarcane Phytophthora isolate, by adding a large quantity of sterilized oats on which the fungus was growing abundantly to this soil, and (4) the seed pieces were planted in a combination of

PLATE II

Arrangement of boxes with sterilized soil artificially infested with Phytophthora, planted with seed pieces of the varieties P.O.J. 213, Co. 290 and C.P. 36-13 and kept under simulated flooding conditions.

treatments 2 and 3.

Since the experiment was set up with sterilized soil, in order to eliminate the possible injury and infection of the seed pieces by other soil microorganisms, only 10 seed pieces of each variety could be used for each treatment. These 10 seed pieces, however, were definitely free of any growth cracks, borer holes or other defects and were used in their entire length.

The seed pieces were planted in November and the boxes were watered frequently, so as to partly simulate flooding conditions often prevailing in the field (Plate II). The canes were dug, split and examined at 3 and 4-1/2 months after planting respectively. Unfortunately, however, the temperature was rather high for the development of Phytophthora rot during the first two months of the experiment but changed later to more favorable temperatures.

Upon examination of the canes, percentages of germinated buds and diseased internodes were calculated. Isolations were made from diseased nodes and internodes in order to obtain a percentage of Phytophthora and Physalospora recovery from these tissues.

Field Tests

In order to test the susceptibility of sugarcane varieties to the Phytophthora isolate, many varieties were planted in the field during the fall of 1957 and 1958. These varieties were represented by several of the P.O.J. and Co. canes, all of the present commercial varieties and several experimental test field varieties. The inoculum was made up by growing the fungus in large quantity on sterilized oats in 2000 ml. Erhlenmyer flasks in the cold room (70° F.) for 14 days. At the time of planting, these oats with the fungus were sprinkled in the furrow so that the seed pieces were surrounded by many grains before being covered up with soil. (Plate III).

The sugarcane varieties tested were:

P.O.J. 36	C.P. 807	C.P. 44-155
P.O.J. 213	C.P. 28-19	C.P. 47-193
P.O.J. 234	C.P. 29-116	C.P. 48-103
	C.P. 29-320	C.P. 51-21
	C.P. 34-120	C.P. 52-68
Co. 281	C.P. 36-13	C.P. 53-1
Co. 290	C.P. 36-105	C.P. 53-15
	C.P. 43-47	C.P. 53-22
	C.P. 44-101	C.P. 53-23
N.Co. 310	C.P. 44-154	C.P. 55-30

The cane to be used was cut the day before planting and the individual replications were tied with thin wire to ease the digging and examination of the seed pieces the following spring. In the 1957-1958 experiment, 5 stalks were used per replication for the infested

PLATE III

Soil infestation in the field through the addition of sterilized oats with the sugarcane Phytophthora isolate, in the furrow with the seed pieces.

soil, while the checks contained 3 stalks each. For the 1958-1959 experiment, these numbers were 4 and 3 respectively, while the following varieties were not repeated that year: C. P. 29-320, C.P. 44-154, C.P. 53-15 and C.P. 53-23.

All the varieties were planted in 3 replications in order to allow examination of the seed pieces at different periods during the following spring, in order to check them for progressive infection and spread of the pathogen. These periods ranged from January 25 - February 23, February 25 - March 20 and March 27 - April 25 during the spring of 1958, while during the spring of 1959, all periods were 3 weeks later than mentioned above, due to very dry weather conditions the previous winter.

After the canes were dug, they were freed of excess soil in the field and taken to the greenhouse to be washed, after which each individual stalk was split lengthwise with a cane knife in order to be examined internally. A count was made of the number of diseased internodes. All nodes, internodes and end pieces suspected of being infected by Phytophthora were then taken into the laboratory to be plated out according to standard procedures used by various investigators (71, 75).

The individual pieces of the stalks were surface sterilized by dipping them into a solution of 0.2 per cent mercuric chloride for about 5 minutes, followed by immersion in a calcium hypochlorite

solution for about 10 minutes. The mercuric chloride was made up by placing 2 tablets of the chemical in 1 liter of water, while the calcium hypochlorite contained 40 gr. of the material in 1 liter of water. Plugs of diseased tissue were removed by the use of a No. 3 (8 mm) cork borer and imbedded in plates of oatmeal agar medium which had previously been acidified with one drop of 50 per cent lactic acid per Petri dish. Each diseased node, internode, bottom and top end of the seed pieces was plated in a separate dish, by using two plugs of the first and four to five plugs of the other tissues respectively. The Petri dishes were then incubated for six days in a constant temperature room at 70° F., after which they were examined and outgrowing organisms recorded.

The same number of stalks of all the varieties used in the 1958-1959 experiment were planted in a second location of the sugarcane experimental farm. Additional inoculum of Phytophthora was also added to this experiment. The canes were not dug for examination, however, but were left in the ground to see whether any appreciable difference in stand would appear the following spring. No shaving or offbaring was practiced in this experiment. Unfortunately, the P.O.J. and Co. varieties in the infested soil were covered up with an additional 3 feet layer of soil from an unforseen ditching project, so that no data could be obtained from those varieties. The stand count was started on April 15, 1959 and was continued at monthly intervals until July 15.

In order to check the amount of Phytophthora seed piece rot under natural conditions, without additional soil infestation, a survey was made in the sugarcane belt of Louisiana during the spring of 1958. Wherever gaps appeared in the rows of plant cane, the ungerminated seed pieces were dug, washed in the greenhouse upon return and then taken into the laboratory for a closer examination. The internodes to be plated were surface sterilized with mercuric chloride and calcium hypochlorite, as described before, after which platings were made on oatmeal agar and all dishes were stored in the cold room (70° F.).

EXPERIMENTAL RESULTS

Induction of Fruiting Structures and Description of the "Sterile" Phytophthora Isolate

Upon examination of the oats, on which the "sterile" Phytophthora isolate was growing abundantly, by scraping a small amount of surface tissue with the fungus and placing it on a slide under the microscope, a large number of oospores was observed (Plate VIII, Fig. 1). This finding stimulated additional examination and when the oat grains, covered with the fungus, were in turn placed in dishes with sterile water, many sporangia and chlamydospores were produced within 48 hours (Plate IV, Fig. 1). It was found, that the number of sporangia was even more abundant, when the oats were placed on wooden rods in large flasks filled with regular tap water instead of sterile water. When this "sterile" isolate was grown on oatmeal agar or on any other culture medium, no fruiting structures were ever observed, with the exception of some chlamydospores in water, which fact confirms the findings of previous workers (71, 75).

Since many investigators have used different words for the same term in the description of an organism, which often resulted in misunderstanding, all terms used in the following description and identification of this Phytophthora isolate are according to the latest standard terminology of this genus as proposed by Blackwell (11).

The young mycelium was typically coenocytic but has been observed to become septate with age. The hyphae are typical of the genus Phytophthora, being very variable in diameter, even along the same hypha, and possessing numerous hyphal swellings. The diameter, excluding the swellings, varies from 1.7-5.1u, with an average of 3.2u.

The sporangia are borne on long sporangiophores (50 - 200u), many measuring over 400u in length (Plate IV; Plate V, A). Only occasional branching of the sporangiophore was observed. The interesting feature observed was that a single sporangiophore had an excessive amount of proliferation which was of the extended type, producing a new sporangium a short distance ahead of the old empty one. As many as 6 empty sporangia have been observed on one sporangiophore. A few proliferations were found to be of the "nested" type (Plate V, B). The sporangiophores were never found to be of the compound sympodial type (P. infestans or P. phaseoli) or of the simple sympodial type characteristic of P. cactorum (11).

The sporangia are ovoid to bluntly ellipsoid in shape and have inconspicuous papillae (Plate V, C). They range in size from 20-39u in width and from 27-56u in length, averaging 27 x 42u (average of 75 measurements). They germinate most frequently by the production of zoospores, while occasionally, also direct germination was observed by the production of one germtube through the germ pore or opened papilla (Plate V, D). Never was any germination with more than one

PLATE IV

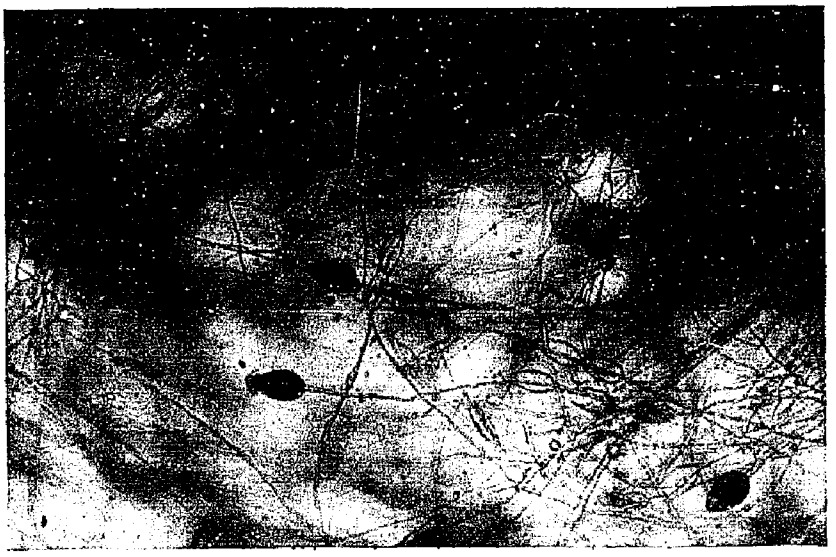
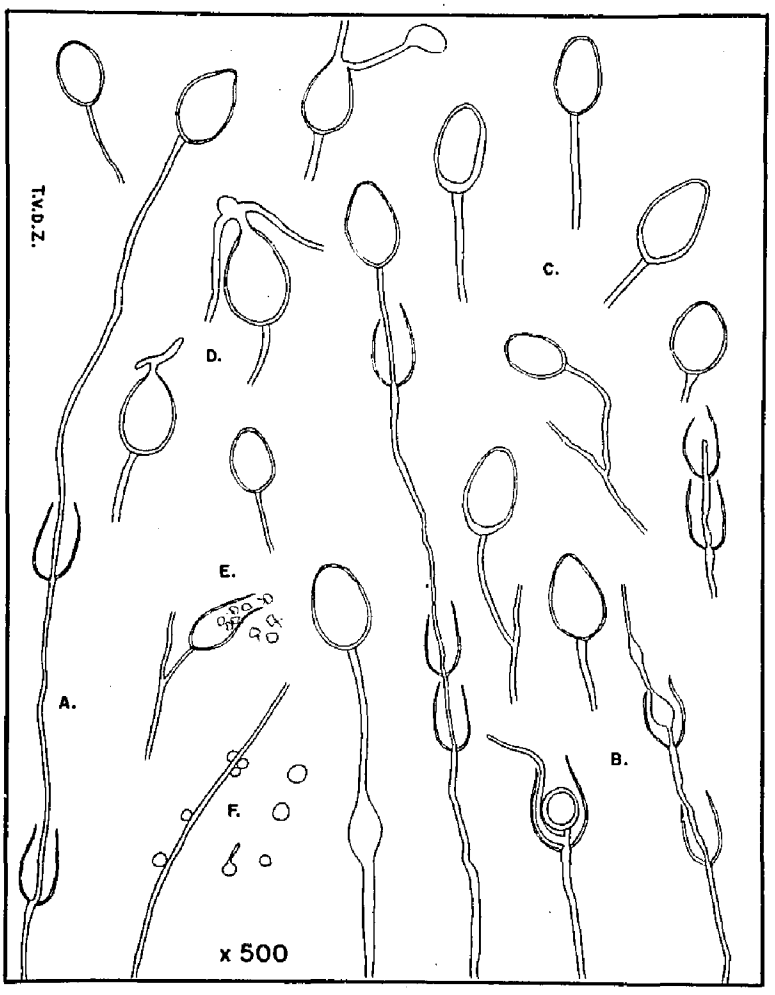


Figure 1

Figure 2

Typical ovoid non-papillate sporangia of P. megasperma on long sporangiophores with abundant proliferation.

PLATE V



Camera lucida drawing of sporangia and zoospores of P. megasperma.

A. typical long sporangiophore
B. proliferation of sporangiophore
C. non-papillate sporangia
D. direct sporangial germination
E. germination by zoospores
F. encysted zoospores

germtube observed, as has been reported to be the case in P. cryptogea (64), P. megasperma (28) and P. terrestris (73).

The liberation of zoospores was often observed and occurred in a manner typical for the genus Phytophthora (Plate IV, Fig. 2), without the production of an emission tube ending in a vesicle into which the undifferentiated mass of protoplasm is discharged, characteristic of the genus Pythium. In a few cases, however, the zoospores were observed to stick momentarily to the mouth of the sporangium in an evanescent "vesicle," before bursting forth all at once, a condition which has also been described for P. cactorum (98). The wall of this vesicle is real thin and is difficult to observe. The number of zoospores from one sporangium varied from 12 to as many as 45. They were irregular in shape at first but became perfectly round after a short swarming period and measured 7-10u (average 9u) upon encystment, usually occurring along the hyphae of the mycelium.

The chlamydospores were typically intercalary and with a few exceptions perfectly spherical in shape, ranging 20-45u (average 36u) in size (average of 100 measurements). They were observed to germinate with 1-7 germtubes, but most commonly with 3-4 germtubes (Plate VI, Fig. 1; Plate VII).

Besides chlamydospores, hyphal swellings were observed in abundance, which in some cases resemble the former very closely. They could be distinguished, however, by the fact that they were not

PLATE VI

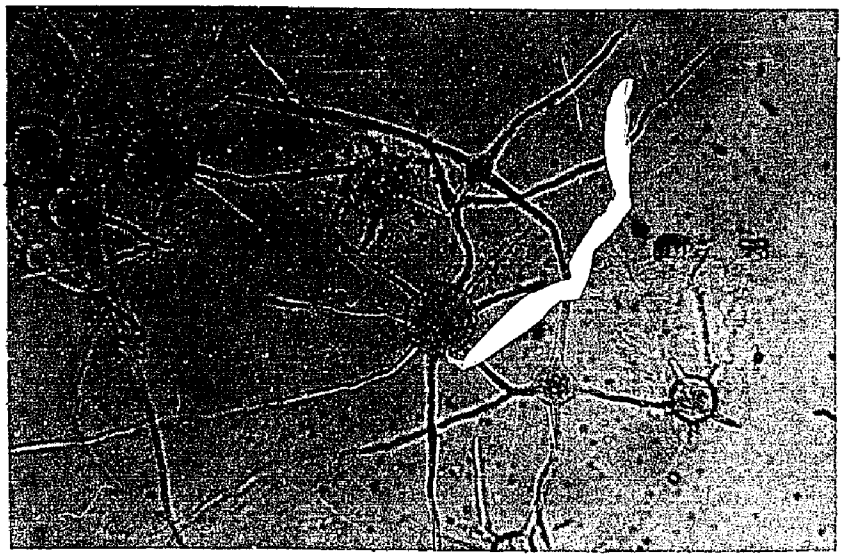
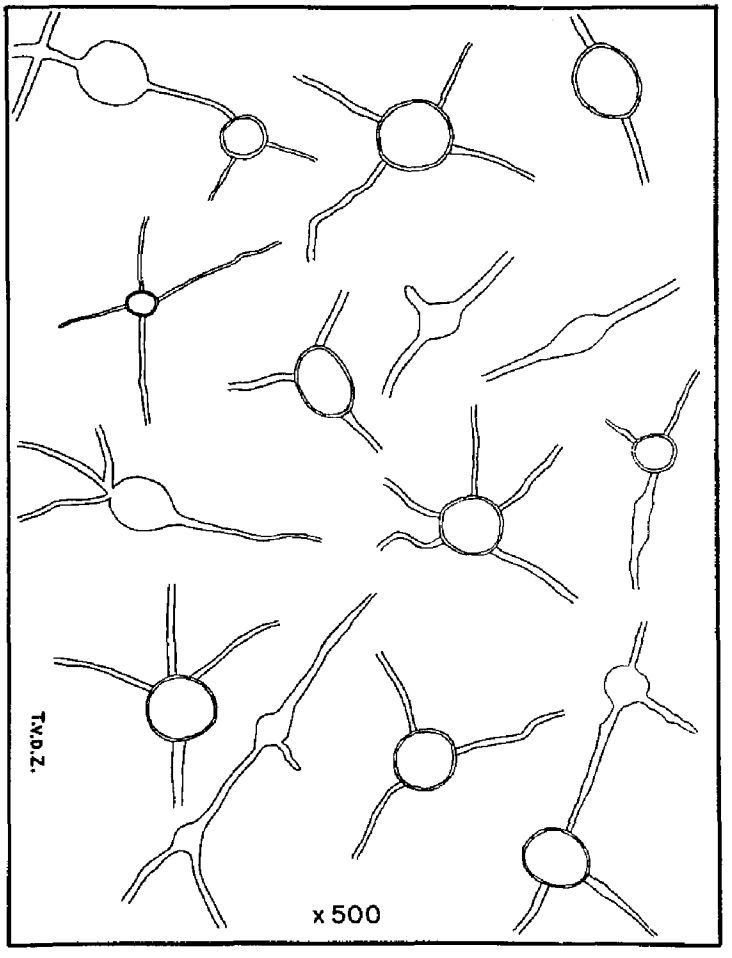


Figure 1

Figure 2

Intercalary chlamydospores and hyphal swellings of P. megasperma.

PLATE VII



Camera lucida drawing of chlamydospores and hyphal swellings of P. megasperma.

cut off from the hyphae by a septum, as was the case for the chlamydospores (Plate VII).

In regard to the sexual fruiting structures, oogonia and oospores were observed in abundance as stated before. All sizes reported for the sexual fruiting structures are an average of 100 measurements. The oogonia were spherical in shape and ranged from 25-41u to 26-42u in size, with an average of 35u. The antheridia were usually paragynously attached to the oogonia but several of these male structures were observed to be amphigynous in nature (Plate IX). In the latter case they were attached closely to the stalk of the oogonium and were often hard to distinguish. The antheridia were from 12-15u in width and from 12-22u in length, and averaged 13 x 15u.

The oospores are perfectly spherical, plerotic and measure from 18-38u to 20-37u (average 31u) in size. They contain a thick endospore, up to 10u in width (Plate VIII, Fig. 2 and 3). Germination of oospores was not observed in this study.

The above mentioned measurements of the Phytophthora isolate from sugarcane confirm those found by previous workers rather closely. Sanchez (71) reported chlamydospores with an average of 38u, while Singh (75) gave the following ranges: sporangia 28-76u, oogonia 13-29u and oospores 10-21u.

PLATE VIII

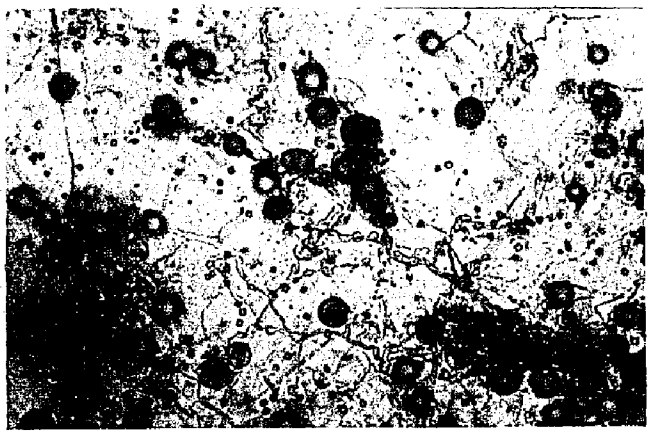


Figure 1

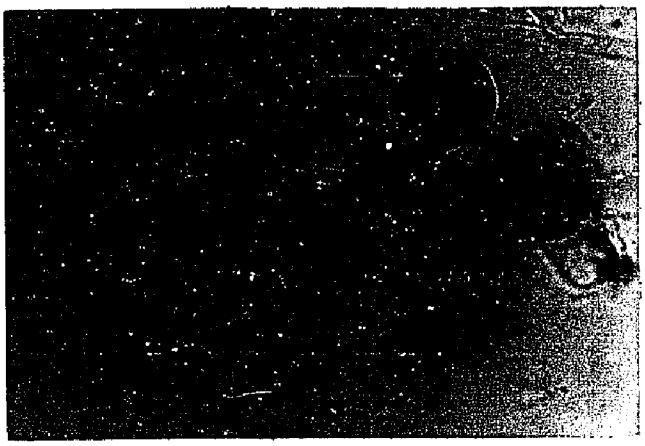
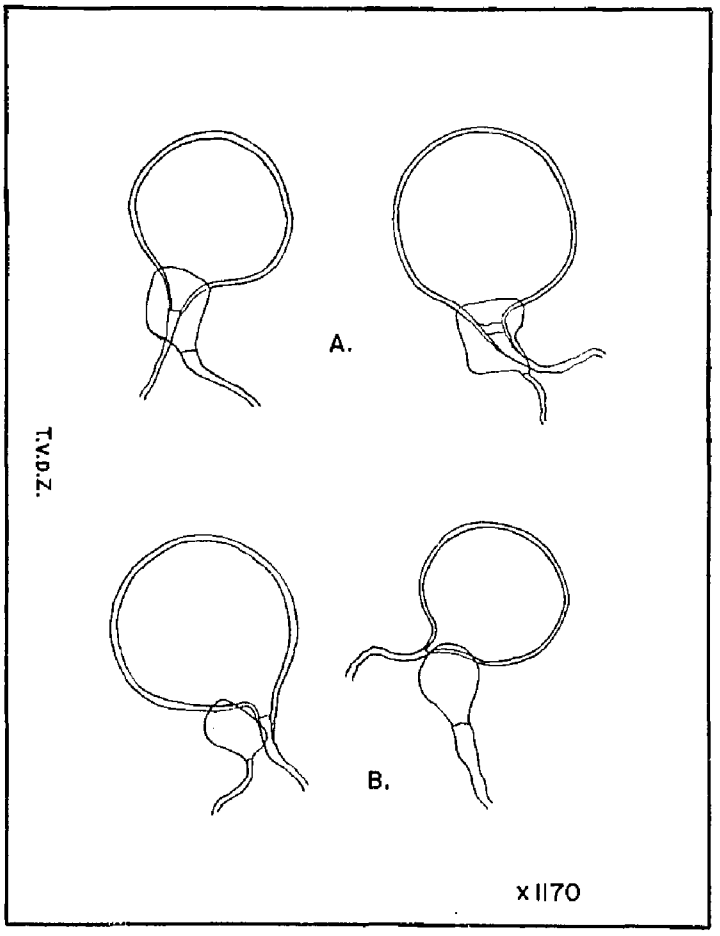


Figure 2

Figure 3

Antheridia, oogonia and oospores of P. megasperma

PLATE IX



Camera lucida drawing of antheridia and oogonia of P. megasperma.

A. amphigynous antheridia
B. paragynous antheridia

Identification of the Principal Phytophthora Species Causing Seed Piece Rot of Sugarcane

The genus Phytophthora was erected by de Bary (25) in 1876, through his studies on the destructive potato blight fungus, Phytophthora infestans. Since that date, many new species have been described in the genus which now totals about 72 species. Several keys have been published of Phytophthora species present in different countries, like those recorded in North America by Wilson (97), in Malaya by Thompson (81), in Argentina by Frezzi (38) and in the British Isles by Waterhouse and Blackwell (93). Some monographic works on the genus, useful for the identification of Phytophthora species, have been published by Rosenbaum (70), Tucker (85) and Leonian (53), while recently, Waterhouse (90) made a compilation of descriptions and illustrations of all previously recorded species of Phytophthora, including figures from the original papers, which work is very valuable for identification purposes, since many of these original publications are not readily available today.

In order to identify the principal Phytophthora species responsible for seed piece rot of sugarcane, a review was made of all known species of the genus Phytophthora. After elimination of all twig, leaf and fruit pathogens from the list of about 72 described species, there are about 20 species left which are known to cause a rotting of roots, corms, tubers or other underground parts of plants. Of these 20 species,

many are known to attack specific hosts only, like P. quininea on cinchona (23), P. primulae on primula and polyanthus (82) and P. richardiae on arum lily (13).

After comparison of typical morphological characteristics of the remaining Phytophthora root pathogens, it was found that the species P. cinnamomi Rands, P. cryptogea Pethyb. & Laff., P. drechsleri Tucker and P. megasperma Drechs. showed the closest resemblance to the Phytophthora isolate from sugarcane. Also P. erythroseptica Pethyb. was used for comparison, since this species had already been described from sugarcane as a less virulent pathogen of seed piece rot (71, 78). A compilation of these characteristics is presented in Table I.

The first reason why the four species mentioned above were selected for comparative studies, is the fact that all species are characterized by having non-papillate sporangia, produced on sporangiophores which proliferate by resuming growth through the base of old evacuated sporangia, to produce new ones some distance ahead (85, 90). In addition, P. megasperma and P. cinnamomi are characterized by the formation of long sporangiophores measuring mostly 50-200u or more in length (28, 90). These three typical sporangial characteristics have also definitely been observed in the Phytophthora isolate from sugarcane. It can also be seen from Table I, that the widths of the sporangia of the sugarcane isolate fall in the range of those given for P. cinnamomi and P. megasperma, the latter

Table I. Comparison of morphological characteristics and measurements of fruiting structures of different Phytophthora species with the unidentified Phytophthora isolate from sugarcane.

Phytophthora species	Authority	Fruiting Structures (Sizes in u) Sporangium	Zoospore	Chlamydospore	Antheridium	Oogonium	Oospore	Reference
P. cinnamomi	Rands	27-39 x 38-84	10-11	terminal 31-50			unknown	(67)
P. erythroseptica	Pethyb.	20 x 32		unknown	amphigynous		29-30	(63)
P. drechsleri	Tucker	15-24 x 24-38		unknown	amphigynous	31	26	(85)
P. cryptogea	Pethyb. & Laff.	17-30 x 24-50	10-15		amphigynous	31	25	(64)
P. megasperma	Drechs.	6-45 x 15-60	10-13	intercalary	paragynous & amphigynous	42-52	37-47	(28)
Phytophthora sp.*	Range:	20-39 x 27-56	7-10	20-45 intercalary	paragynous & amphigynous	25-42	18-38	present study
	Average:	27 x 42	9	36	13 x 15	35	31	

*isolate from sugarcane

having a wider range however. The sporangia of P. cryptogea and P. drechsleri on the other hand are much smaller in width (aver. 21u).

P. cinnamomi is distinguished from the other species by the production of typical terminal chlamydospores, while those of P. megasperma and the sugarcane isolate are definitely intercalary only. Upon examination of the sugarcane isolate, Zentmyer (100) concluded that it was definitely not P. cinnamomi. Chlamydospores have not been reported from P. erythroseptica, P. drechsleri or P. cryptogea. Also the hyphal swellings of P. cinnamomi are different, being produced as large spherical bodies in crowded clusters, as compared to the smaller single swellings of P. cryptogea and P. erythroseptica, which are usually produced in chains (11, 85). The latter have also been termed "sphaero-conidia" by Lafferty and Pethybridge (49). In the sugarcane isolate, on the other hand, the hyphal swellings are much more isolated and are smaller in size than the swellings of P. cinnamomi, but larger than those of P. cryptogea.

In comparing the sexual fruiting structures of these Phytophthora species, it will be noted from Table I, that P. erythroseptica, P. drechsleri and P. cryptogea produce only amphigynous antheridia, while those of P. megasperma have been found to be of both the amphigynous and paragynous type. Drechsler (28) reported in the original description of this species, that in 35 out of 100 cases the antheridia were amphigynous, the rest being paragynous. In the latter

case, they are usually attached near the stalk of the oogonium, which was also found to be true in the present study of the sugarcane isolate.

Oospores have seldom been observed in P. cinnamomi. Ashby (6), however, did find some in old oatmeal agar cultures. P. erythroseptica, P. drechsleri and P. cryptogea have smaller oogonia and consequently smaller oospores than P. megasperma. The sugarcane isolate, however, has oogonia and oospores slightly larger in size than the three species just mentioned, but also smaller than P. megasperma. The isolate from sugarcane was also found to resemble P. megasperma in having a rather thick endospore in the oospore.

At this point, it can be seen without much doubt, that the "sterile" Phytophthora isolate from sugarcane is morphologically distinctly different from P. cinnamomi, P. erythroseptica, P. drechsleri and P. cryptogea and resembles P. megasperma most. Because of its non-papillate sporangia, produced on long sporangiophores with excessive proliferation, combined with the formation of intercalary chlamydospores, paragynous antheridia and oospores with a thick endospore, the Phytophthora isolate from sugarcane is morphologically identified as Phytophthora megasperma Drechsler.

Cultures of the sugarcane isolate were sent for identification to two of the world's leading authorities on the genus Phytophthora, Dr. J. T. Barrett in Berkeley, California and Miss Grace M. Waterhouse at the Commonwealth Mycological Institute in Kew, England. In his

examination of this sugarcane isolate, Barrett (9) also found many paragynous antheridia, numerous chlamydospores and abundant proliferation of the sporangia and suggested to call the isolate Phytophthora miyabeana (Pythiomorpha miyabeana Ito & Nagai).

The genus Pythiomorpha was erected by Petersen (13) in 1910, with one species representing, P. gonapodyides, on account of the first observance of sporangial proliferation. In 1931, Ito and Nagai (91) reported two species of Pythiomorpha from rice seedlings in Japan, one being P. miyabeana. Since then, Buisman (13) included P. gonapodyides in the genus Phytophthora as Phytophthora gonapodyides (Petersen) Buism., while Waterhouse (91) suggested recently to place Pythiomorpha miyabeana in synonymy with Phytophthora megasperma Drechs. She compared a culture of Pythiomorpha miyabeana with an isolate of P. megasperma described by Tompkins and his co-workers (84) from a root rot of cauliflower in California and also with Phytophthora isolates reported from soybean roots in Ontario and from peach roots in New South Wales, and came to the conclusion that there was no appreciable difference between them except for smaller sized oogonia and consequently smaller oospores. Tompkins et al. (84) reported oogonia with a range of 36-52u (average 43u) as compared with the average 47u of the type culture identified by Drechsler (28), while Pythiomorpha miyabeana had oogonia with an average

diameter of 38u, ranging from 32-46u (91). These latter measurements also fit the range and average of the oogonia of the sugarcane isolate.

Upon examination of the Phytophthora isolate from sugarcane, Waterhouse (92) stated that also this isolate seemed to be a small-spored form of Phytophthora megasperma, which conclusion in turn confirms independently Barrett's identification of the sugarcane isolate mentioned before.

Cultures of the P. megasperma isolate from sugarcane have been sent for permanent deposit in the American Type Culture Collection (A.T.C.C.), Washington, D. C. and in the Centraal Bureau voor Schimmelcultures (C.B.S.), Baarn, The Netherlands.

Growth-Temperature Relation Studies

The effect of temperature on the growth of the sugarcane Phytophthora isolate was measured by recording every other day the diameter of a colony grown on potato dextrose agar. These data were compared with those obtained from other Phytophthora species tested in a similar manner. The amount of growth of the different species after 5 days is shown in Graph 1. It can be seen immediately that P. cinnamomi, P. erythroseptica and P. drechsleri grew faster than the other species at the temperature range 15° - 30° C. P. cryptogea, on the other hand, gave the least amount of growth at all temperatures tested, while P. megasperma grew closely parallel to the Phytophthora isolate from sugarcane, with a little more growth than the latter at temperatures 15° - 30° C.

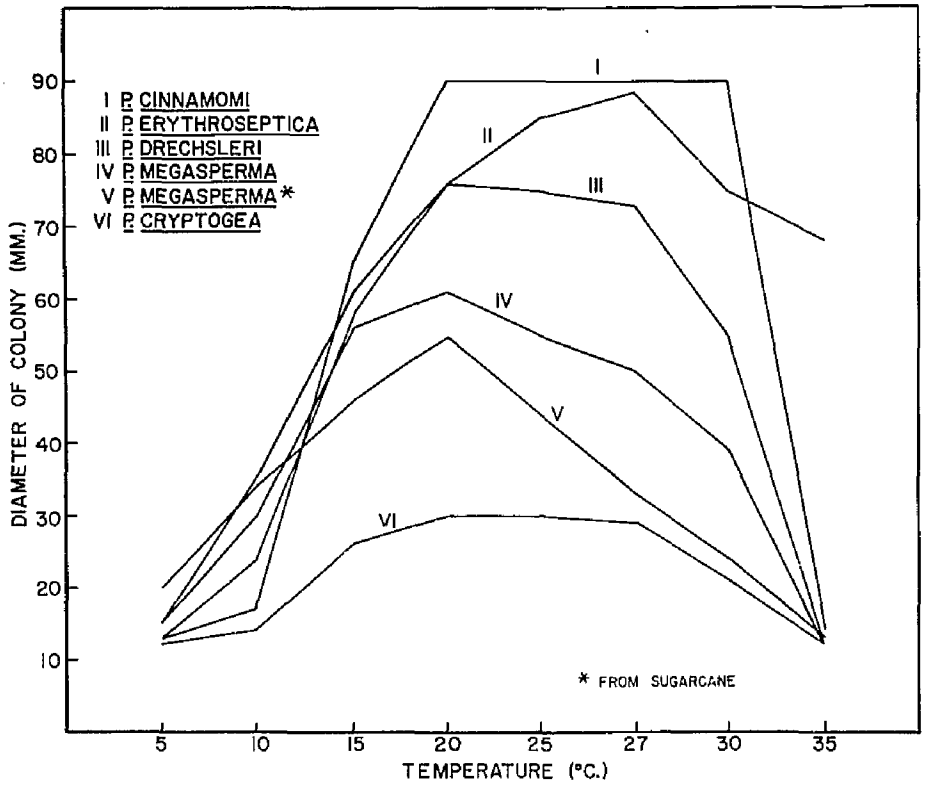
The optimum temperature of the sugarcane isolate was found repeatedly to be between 20° and 22° C., while in plate culture this isolate grew differently from all other species tested, in that the fungus grew mostly submerged in the agar with very little aerial mycelium (Plate X). All species tested were found to have a similar minimum and maximum temperature, with the exception of P. erythroseptica which gave a good amount of growth at 35° C.

These results confirm rather closely those found by other investigators. Tucker (85) reported in his temperature studies of Phytophthora species, that P. cinnamomi grew best at the temperature range 20°-30° C.,

with an optimum at 25°-27.5° C. Wager (88), who studied two strains of P. cinnamomi, one from avocado in South Africa and the other from citrus in Brazil, stated that the former had its optimum at 25° C. and the latter between 28° and 31° C. For P. drechsleri and P. erythroseptica, Tucker (85) found the optimum growth to be 27.5°-32° C. and 27.5° C. respectively, but reported the latter species as having no growth at 35° C. The data obtained in the present temperature study, in respect to P. cryptogea, however, confirm very closely those reported by Erwin (34) and Middleton (60) and his associates, who reported minimum temperatures of 8° and 1° C., optimum 25° and 22°-25° C., and maximum 30°-33° and 31°-34° C. respectively.

In respect to P. megasperma, Jones (46) found in his study on a root rot of sweetclover caused by this species, that in inoculation experiments the disease was equal in severity over a soil temperature range of 10° to 24° C. Wager (89) stated that P. megasperma from orange roots had an optimum radial growth in culture at 19° C., with a range from 4°-28° C. Singh (75), however, reported in his study of sugarcane seed piece rot that the "sterile" Phytophthora isolate grew at its optimum at 11° C. in plate culture.

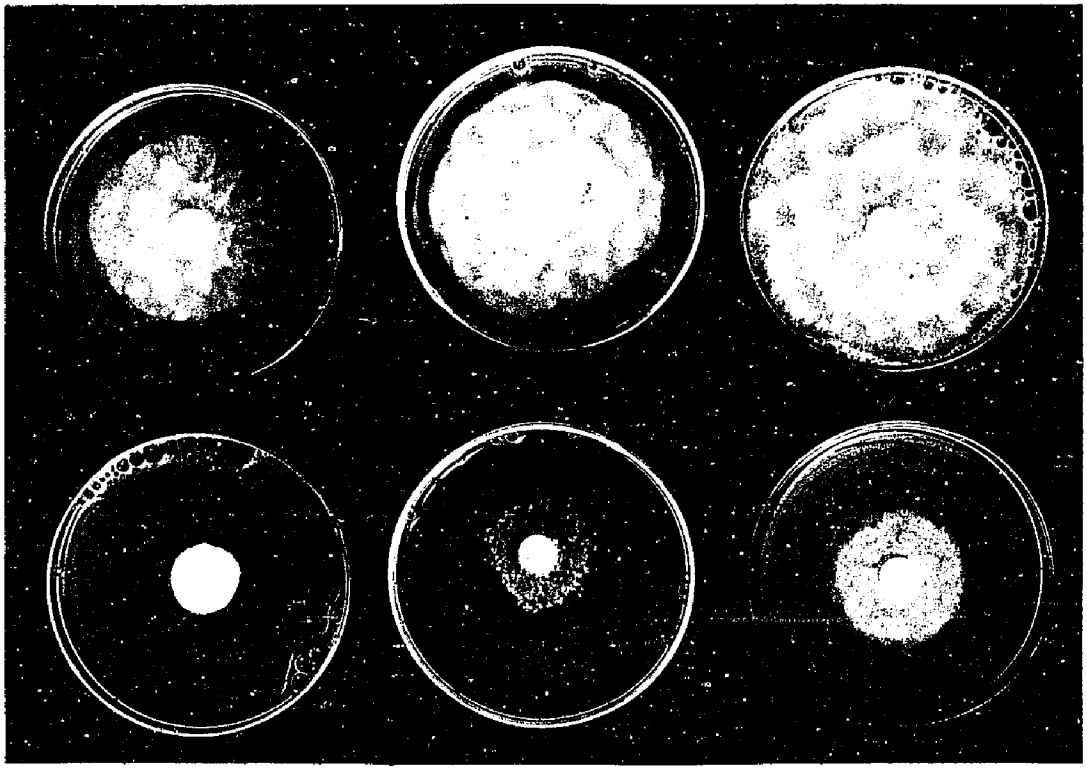
An interesting feature of this temperature study was found, when the amount of radial growth was measured after 10 and 30 days for the minimum and maximum temperatures respectively. These data are presented in Table II. Both P. megasperma and the sugarcane isolate gave



Graph 1

Comparison of average radial growth of different Phytophthora species with P. megasperma from sugarcane, in plate cultures on potato dextrose agar, incubated 6 days at various temperatures.

PLATE X



Comparison of average radial growth of different Phytophthora species with P. megasperma from sugarcane, growing on potato dextrose agar, incubated 5 days at 20° C. From left to right, at top: P. drechsleri, P. erythroseptica and P. cinnamomi; at bottom: P. cryptogea, P. megasperma (from sugarcane) and P. megasperma.

Table II. Comparison of average diameters of mycelial growth of different Phytophthora species with P. megasperma from sugarcane in plate cultures on potato dextrose agar incubated 10 and 30 days at various temperatures.

Phytophthora species	Average Colony Diameter (mm)										
	After 10 days								After 30 days		
	5°	10°	15°	20°	25°	27°	30°	35°	5°	30°	35°
P. cinnamomi	13	46	90	90	90	90	90	14	16	90	14
P. erythroseptica	15	53	90	90	90	90	90	84	30	90	90
P. drechsleri	19	65	90	90	90	88	72	12	34	76	12
P. megasperma	21	70	90	90	90	74	55	12	50	80	12
P. megasperma*	26	59	82	90	72	39	24	13	51	30	13
P. cryptogea	12	30	46	50	49	44	30	12	17	50	12

*isolate from sugarcane

a fair amount of growth after 1 month incubation at 5° C., as compared to the other 4 Phytophthora species tested. This finding shows again the close identity of the sugarcane Phytophthora isolate to the type culture of P. megasperma, and also that this species seems to be well capable of survival and growth at this low temperature and consequently at the range of 5°-20° C., which point is in conformity with the fact that Phytophthora seed piece rot is most severe when winter temperatures are low.

The growth-temperature relation of P. erythroseptica and P. megasperma in comparison with the sugarcane isolate was tested on 5 additional culture media at temperatures of 10°, 20° and 32° C. After an incubation period of 6 days, the colony diameters were recorded, which data are shown in Table III. It was found that the two mineral media, Czapek and Fries agar, were unfavorable for growth of the Phytophthora isolates. Oatmeal, cornmeal and lima bean agar were found to give about the same amount of growth as did potato dextrose agar . P. megasperma grew again closely parallel to the sugarcane isolate on all media used.

Upon examination of the inoculated fruit in the moist chambers, it was found that both P. megasperma and the sugarcane Phytophthora isolate were non-pathogenic to eggplant, weakly pathogenic to apples, while both produced a slow, brown leathery rot in oranges. Gooi and Tassinari (39) reported from Italy, that P. megasperma, isolated from

Table III. Comparison of average diameters of mycelial growth of P. erythroseptica and P. megasperma with P. megasperma from sugarcane, in plate cultures on different media incubated 6 days at 10°, 20° and 32° C.

Phytophthora species	Average Colony Diameter (mm)														
	Oatmeal Agar			Cornmeal Agar			Lima Bean Agar			Czapek Agar			Fries Agar		
	10°	20°	32°	10°	20°	32°	10°	20°	32°	10°	20°	32°	10°	20°	32°
P. erythroseptica	48	89	90	33	90	83	38	90	90	18	23	29	11	28	29
P. megasperma	20	64	10	13	24	10	18	60	10	14	18	10	15	17	10
P. megasperma*	39	74	32	12	16	22	32	68	20	19	33	13	19	22	14

*isolate from sugarcane

peach trees, did also infect apples in inoculation experiments, while Wager (89) found, upon inoculation of oranges and lemons with P. megasperma, the same type of brown rot as was observed in the present study. These comparisons seem to indicate again the close relationship of the sugarcane Phytophthora isolate to P. megasperma.

In the pathogenicity experiment, in which P. megasperma, P. erythroseptica and the sugarcane Phytophthora isolate were compared for their ability to cause seed piece rot, the canes were dug for examination 15 days after inoculation and the average length of the diseased area in the seed pieces was measured. The data are presented in Table IV. It was found in this test, that the sugarcane isolate was about twice as virulent as P. erythroseptica, based on the size of

Table IV. Comparative pathogenicity of P. erythroseptica and P. megasperma with P. megasperma from sugarcane, in seed pieces of the varieties Co. 290 and C.P. 34-120, 15 days after inoculation.

Sugarcane Variety	Length of Diseased Area (Cm.)		
	P. erythroseptica	P. megasperma	P. megasperma*
Co. 290			
Check	0.0	0.0	0.0
Inoculated	3.7	5.2	6.2
C.P. 34-120			
Check	0.0	0.0	0.0
Inoculated	4.4	6.9	8.8

*isolate from sugarcane.

the diseased area in the internodes. This confirms the results reported by previous investigators of Phytophthora seed piece rot (71, 75, 80). The type culture of P. megasperma was also observed to be more pathogenic than P. erythroseptica, but only slightly less than the sugarcane isolate. In comparing all isolates on both sugarcane varieties, Co. 290 was found to be less susceptible to the Phytophthora cultures than was C.P. 34-120. The diseased tissue of the latter variety had the typical pink discoloration, while in the former the tissue was only watersoaked in appearance.

Basic Seed Piece Infection Studies

When the first seed pieces were dug from the infested soil, 2 weeks after planting, it was noticed immediately that several of the yet ungerminated root primordia and some of the buds were swollen, seemingly infected and showing a distinct red discoloration. A few weeks later, many more of these tissues had become infected, including also some of the leaf scars (Plate XI and XII).

In order to keep an accurate account of the probable increase of infection in later examinations, a count was made of the total number of root primordia and buds and also of the number of infected ones of the two varieties tested in this experiment, N.Co. 310 and C.P. 36-13. The average number of root primordia of one node of these varieties was found to be 65 for N.Co. 310 and 50 for C.P. 36-13. At the first examination of the seed pieces, two weeks after planting, it was found that out of 1300 root primordia (20 eyes) of N.Co. 310 and 1000 root primordia (20 eyes) of C.P. 36-13, 82 and 52 had become infected respectively. Besides this infection, also 6 buds of N.Co. 310 and 7 of C.P. 36-13 were found to be diseased. These examinations were continued at 3, 5, 7 and 9 weeks after planting and the percentage infection determined can be found in Table V.

It can be seen from this table that the percentage infection of both the root primordia and the buds increased successively, reaching 55.5 and 70.0 per cent respectively for N.Co. 310 and 67.9 and 80.0 per

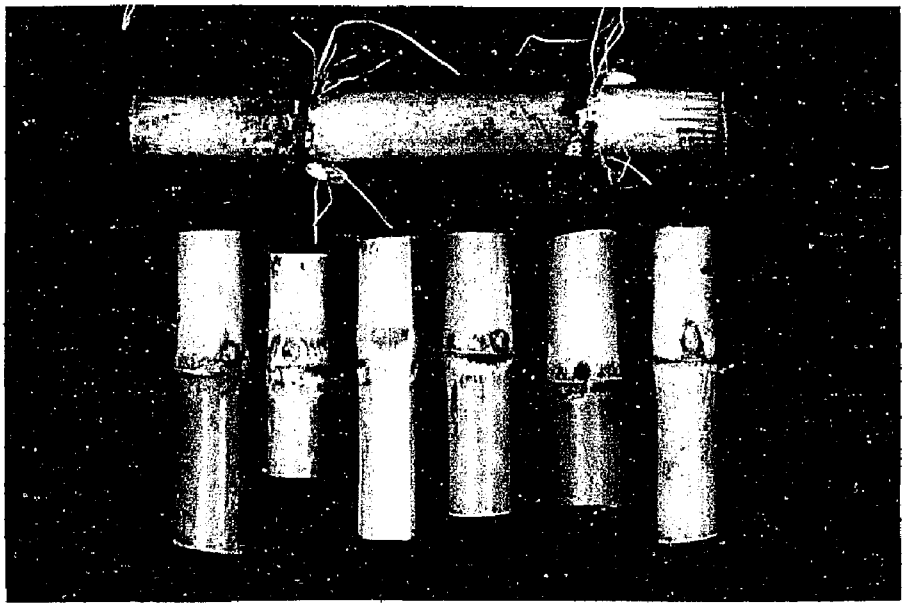
cent for C.P. 36-13 after 9 weeks. The pathogen, P. megasperma was consistently re-isolated from the infected tissues during the 5 successive examinations. It was also found in this study, that there was always a higher percentage of root primordia and buds infected of the variety C.P. 36-13 than of N.Co. 310. None of the root primordia and buds of the seed pieces used for the checks were ever found to be infected and plating of these healthy tissues only resulted in some cultures of Fusarium.

By plating out the internal tissue of each node, a percentage was obtained of the amount of infection in these tissues. It can be seen from the table that this percentage was always lower than the percentage bud infection, up to the examination at 7 weeks after planting. This can be explained by the fact that in the early stage of seed piece infection the fungus had penetrated the nodal region externally, but had not spread far enough into the seed piece, to be recovered internally in that particular node.

At 9 weeks after planting, the fungus had not spread much farther than the nodal region and the internode of each seed piece was still healthy and uninvaded.

The most interesting phenomenon observed in this basic seed piece infection study was the fact that the root primordia and buds of the seed pieces planted in infested soil, which had escaped infection by the pathogen, had not germinated at all, while those in the checks

PLATE XI



External infection of root primordia, buds and leafscars of seed pieces of the variety C.P. 36-13, planted in sterilized soil artificially infested with P. megasperma. Top seed piece from uninfested soil.

Note: Third seed piece from left shows early spread of pathogen into internal cane tissue.

PLATE XII

External infection of nodal regions of seed pieces of the varieties C.P. 36-13 (left) and N.Co. 310 (right), 7 weeks after planting in sterilized soil, artificially infested with P. megasperma. Checks at extreme left and right.
Note: Complete lack of germination of the seed pieces planted in infested soil.

Table V. Percentage infection of root primordia and buds and percentage bud germination of seed pieces of the varieties N.Co. 310 and C.P. 36-13, planted in sterilized soil, artificially infested with P. megasperma.

Time After Planting	Sugarcane Variety	Total No. Single Eyes Planted		Percentage Infection						Percentage Bud Germination	
				Root Primordia		Buds		Internal Nodal Tissue			
		Check	Infest.	Check	Infest.	Check	Infest.	Check	Infest.	Check	Infest.
2 weeks	N.Co. 310	10	20	0.0	6.3	0.0	30.0	0.0	25.0	0.0	0.0
	C.P.36-13	10	20	0.0	5.2	0.0	35.0	0.0	15.0	0.0	0.0
3 weeks	N.Co. 310	10	20	0.0	12.0	0.0	35.0	0.0	20.0	25.0	0.0
	C.P.36-13	10	20	0.0	17.1	0.0	40.0	0.0	25.0	30.0	0.0
5 weeks	N.Co. 310	10	20	0.0	22.6	0.0	55.0	0.0	55.0	30.0	0.0
	C.P.36-13	10	20	0.0	23.7	0.0	60.0	0.0	50.0	50.0	0.0
7 weeks	N.Co. 310	10	20	0.0	41.7	0.0	50.0	0.0	90.0	70.0	0.0
	C.P.36-13	10	20	0.0	51.6	0.0	75.0	0.0	70.0	70.0	0.0
9 weeks	N.Co. 310	10	20	0.0	55.5	0.0	70.0	0.0	100.0	70.0	0.0
	C.P.36-13	10	20	0.0	67.9	0.0	80.0	0.0	85.0	80.0	0.0

started germinating 3 weeks after planting. After 7 weeks, both varieties planted in non-infested soil had 70.0 per cent of the buds germinated, while their root system was quite abundant (Plate XII). The variety C.P. 36-13, however, was found to be somewhat advanced over N.Co. 310 in the total amount of shoot and root development.

Preliminary experiments, in which seed pieces were placed in flasks, with one end submerged in a filtered culture liquid of P. megasperma, did give some indication that a toxin might be produced by the fungus. Additional experimentation will undoubtedly be necessary to test this hypothesis.

In order to study the rate of spread of Phytophthora in sugarcane seed pieces, the fungus was placed inside the cane as described before, after which the seed pieces were planted in crocks with sterilized soil. Five successive examinations were made of the seed pieces, each testing consisting of 20 inoculated and 10 check seed pieces.

The first examination was made 10 days after planting, and upon splitting of the seed pieces a good picture was obtained of the spread of the pathogen. The internodal tissue in the vicinity of the oat grain containing the fungus, had a watersoaked appearance while the tissue at the extreme edges of the infected area had a reddish pink discoloration. The total length of this diseased area was measured, while a note was made of the discoloration, which procedure was repeated in the later examinations at 15, 20, 30 and 50 days after inoculation. These data

are presented in Table VI.

At 15 days after inoculation of the seed pieces, the watersoaked area had become twice as large, while the total amount of discoloration had about reached the nodal tissues of the seed pieces. After 20 days, the watersoaked area had changed to a light purple color, which indicated a start of actual tissue deterioration (Plate XIII).

At 30 days after inoculation, this purple color had become deeper in tint, accompanied by a strong ether-like odor, while after 50 days the edges of the diseased area had spread well past the nodal regions and into the adjacent internodes of the seed pieces. The tissue of the original internode had deteriorated to a great extent, resulting in a large cavity. It was also noticed that this purple discoloration and breakdown of the internodal tissue occurred faster in the variety N.Co. 310 than in C.P. 36-13 (Plate XIV).

The Phytophthora isolate was consistently re-isolated from the watersoaked and reddish discolored areas of the seed pieces, but hardly ever from the areas with the typical purple discoloration, which fact will be discussed in more detail in the last chapters.

In contrast to the finding mentioned before, that the uninfected root primordia and buds of the seed pieces planted in infested soil, never germinated, it was found in this experiment that some germination did occur. The amount of germination, however, of these inoculated seed pieces 50 days after planting, was only 20.0 per cent for N.Co. 310 and 35.0 per cent for C.P. 36-13, as compared to 60.0 per cent and 80.0 per cent for their respective checks.

Table VI. Progressive spread of Phytophthora in seed pieces and percentage bud germination of the varieties C.P. 36-13 and N.Co. 310, inoculated with P. megasperma.

Time After Inoculation	Number Internodes Planted		Aver. Spread of Phytophthora (in cm.)	Appearance of Diseased Area		% Bud Germination			
						C.P. 36-13		N.Co. 310	
	Check	Inoc.		Center	Edge	Check	Inoc.	Check	Inoc.
10 days	10	20	8.0	watersoaked	Pink	0.0	0.0	0.0	0.0
15 days	10	20	12.0	watersoaked	Reddish Pink	10.0	0.0	0.0	0.0
20 days	10	20	14.0	light purple	Red	20.0	0.0	30.0	5.0
30 days	10	20	16.0	purple	Red	50.0	10.0	50.0	15.0
50 days	10	20	20.0	deep purple	Reddish Brown	80.0	35.0	60.0	20.0

PLATE XIII

Figure 1

Figure 2

Internal spread of Phytophthora in seed pieces of the varieties C.P. 36-13 (top) and N.Co. 310 (bottom), artificially inoculated with P. megasperma. From left to right: check; 20 and 30 days after inoculation.

PLATE XIV

Comparison of the severity of Phytophthora rot in seed pieces of the varieties C.P. 36-13 (left) and N.Co. 310 (right), 30 days after inoculation with P. megasperma. Checks at extreme left and right.

Comparative Susceptibility of the Varieties P.O.J. 213, Co. 290 and C.P. 36-13 to Red Rot and Phytophthora Rot

This experiment was set up in order to get some idea of the relative susceptibility of the varieties P.O.J. 213, Co. 290 and C.P. 36-13 to red rot and Phytophthora rot. The first two varieties were of much importance in the variety program of the Louisiana sugar industry until the 1930's and 1940's respectively, when both varieties were replaced by the better hybrids produced at Canal Point, Florida. Since at that time, P.O.J. 213 and Co. 290 were found to be very susceptible to red rot, while Phytophthora rot was not known in those days, it was thought to be of interest to test these two varieties in comparison with C.P. 36-13, the variety that showed such poor stands in 1947 and 1948, and from which Phytophthora was isolated in abundance, which in turn was considered to be the chief cause of the stand failure.

The seed pieces, used as entire stalks of about 4 feet in length, were planted in the boxes with sterilized soil and according to the required treatment, as described before. Half of the stalks were dug and split for examination 3 months after planting and the other half after 4-1/2 months. The data of these two examinations were combined and are presented in Table VII.

From these data, a comparison can be made between the percentage diseased internodes and the percentage bud germination. The checks for all three varieties had the lowest percentage of diseased internodes

Table VII. Comparative pathogenicity of *Phytophthora megasperma* and *Physalospora tucumanensis* in seed pieces of the varieties P.O.J. 213, Co. 290 and C.P. 36-13.

Sugarcane Variety	Treatment	No. Intern. Planted	Intern. Diseased No.	Intern. Diseased %	% Bud Germin.	No. Shoots Above Ground	No. Isolations Made From Nodes	No. Isolations Made From Intern.	% Culture Obtained Phytophthora Nodes	% Culture Obtained Phytophthora Intern.	% Culture Obtained Physalospora Nodes	% Culture Obtained Physalospora Intern.
P.O.J. 213	I	59	3	5.1	52.8	6	4	12	0.0	0.0	75.0	8.3
	II	58	40	69.0	29.4	2	32	63	0.0	0.0	43.8	15.9
	III	63	10	15.9	42.3	3	12	41	25.0	22.0	0.0	0.0
	IV	61	50	82.0	20.0	0	65	123	43.1	47.2	6.2	0.8
Co. 290	I	57	0	0.0	46.2	10	4	8	0.0	0.0	100.0	75.0
	II	57	46	80.7	16.7	3	45	123	0.0	0.0	17.8	5.0
	III	58	6	10.3	39.4	8	18	40	66.7	35.0	33.3	27.5
	IV	57	49	86.0	9.2	0	72	135	38.9	30.4	0.0	7.5
C.P. 36-13	I	53	0	0.0	59.7	12	0	0	0.0	0.0	0.0	0.0
	II	60	37	61.7	34.8	3	35	90	0.0	0.0	31.4	6.7
	III	57	22	38.6	29.4	2	32	55	71.9	76.4	0.0	0.0
	IV	61	49	80.3	20.0	0	82	154	43.9	36.4	8.5	4.5

I = Check
II = seed pieces inoculated with *Physalospora tucumanensis*
III = seed pieces planted in soil infested with *Phytophthora megasperma*
IV = combination of II and III

(0.0 - 5.1%) and the highest percentage germination of the buds, ranging from 46.2 per cent for Co. 290 to 59.7 per cent for C.P. 36-13. Also the number of shoots appearing above the ground at the second examination of the seed pieces, 4-1/2 months after planting, is in close correlation with the percentage bud germination.

As was to be expected, C.P. 36-13 was found to be the most susceptible variety to Phytophthora rot, while Co. 290 was the worst in respect to red rot. In the individual red rot (II) and Phytophthora rot (III) treatments in this experiment, C.P. 36-13 resulted in 61.7 per cent (II) and 38.6 per cent (III) of the internodes diseased, while Co. 290 had 80.7 per cent (II) and 10.3 per cent (III) diseased internodes. The percentage diseased internodes for the variety P.O.J. 213 fell in between those just reported for Co. 290 and C.P. 36-13, for both the red rot and Phytophthora rot treatments. The recovery of the percentage Phytophthora cultures from diseased tissue, however, was considerably lower for P.O.J. 213 than for either Co. 290 or C.P. 36-13.

In order to be more certain of the actual presence of the fungi causing red rot and Phytophthora rot in these diseased seed pieces, isolations were made of the diseased nodes and internodes of all four treatments. The results are shown in the last four columns of Table VII, and the calculations are expressed as a percentage, on the basis of the number of pluggings or isolations made from nodes and internodes.

It can be seen again, that the highest percentage of Phytophthora

cultures was obtained from the Phytophthora treatment (III) of the variety C.P. 36-13, for both nodal and internodal tissues (71.9% and 76.4% respectively). Physalospora tucumanensis was seldom isolated from any of the tissues of C.P. 36-13, except for 31.4 per cent of the isolations made from the internal nodal regions.

In the varieties Co. 290 and P.O.J. 213, more cultures of Physalospora were isolated from the nodal regions than from the internodal tissues. The presence of red rot in the check (I) and Phytophthora treatment (III), is felt to be due to natural infection of the cane, while the low recovery of the red rot fungus from the internodes of these varieties is thought to be caused by too much deterioration of the cane tissue, consequently resulting in the death of the fungus.

Varietal Field Testing

The testing of varieties in respect to Phytophthora rot was carried out at the L.S.U. Experiment Station Farm during the cane seasons 1957-1958 and 1958-1959. The seed pieces were planted in the early part of October and examination for the presence of Phytophthora rot was started in early February of each spring season and was continued for several months thereafter. When the seed pieces were brought in the greenhouse and were freed of excess soil, the percentage bud germination was determined by an examination of each bud. After finishing that part of the examination, every stalk of cane was cut lengthwise and a count was made of the total number of diseased internodes observed. These figures were placed on a percentage basis of the total number of internodes originally planted for each variety. The data for both years are presented in the first four columns of Table VIII.

After the canes were examined, all diseased nodes and internodes which had not deteriorated too much were brought into the laboratory for plating. After incubation for 6 days in a constant temperature room (70° F.), the number of Phytophthora and Physalospora cultures was counted and placed on a percentage basis of the number of pluggings plated, which data are shown in the last four columns of Table VIII.

The data of the three successive examinations and platings carried out each spring season are combined in this large tabulation. It was found during these examinations, that in the spring of 1958 the

amount of nodal infection and internodal discoloration increased very rapidly. Plate XV shows typical nodal infections in a seed piece of the variety C.P. 53-23, as was observed during the earliest examination (February) of the canes in 1958. Plate XVI shows more advanced spread of Phytophthora in seed pieces of the variety C.P. 52-68, this high incidence of the disease is thought to be due to very favorable weather conditions for development of Phytophthora rot during the months right after planting the seed pieces and the late cool spring the following year. This visual observation was later confirmed by the number of Phytophthora cultures obtained from the diseased seed pieces. From October to February (1957-1958), the average temperature ranged from 65°-47° F. (19°-8° C.), while there was an average amount of rainfall of 4.4 inches for that period of time. Due to this low temperature weather and high rainfall during the fall of 1957, natural infection as evidenced by the invasion of the check seed pieces which were planted in soil not additionally infested with P. megasperma, gave fair numbers of Phytophthora cultures upon isolation of the canes.

Taking all the data combined, and comparing each variety planted in infested and non-infested soil, it was found that many varieties had an appreciably higher percentage of diseased internodes, accompanied by a lower percentage bud germination in the infested soil than in the non-infested soil. This difference was found to be even larger, by comparing the percentages of Phytophthora cultures obtained from

Table VIII. The occurrence of Phytophthora and Physalospora in diseased nodes and internodes of seed pieces of many past and present commercial sugarcane varieties and some unreleased experimental test field varieties, planted in soil artificially infested with P. megasperma under field conditions, examined during the spring seasons of 1958 and 1959.

Sugarcane Variety	Year Examined	Treatment	No. Intern. Planted	Intern. Diseased No.	Intern. Diseased %	% Bud Germin.	No. Isolations Made From Nodes	No. Isolations Made From Intern.	% Cultures Obtained Phytophthora Nodes	% Cultures Obtained Phytophthora Intern.	% Cultures Obtained Physalospora Nodes	% Cultures Obtained Physalospora Intern.
P.O.J. 36	1958	Check	78	24	30.7	18.9	7	22	28.6	13.6	71.4	36.4
		Infest.	295	100	33.9	12.3	58	131	67.2	58.8	5.2	1.5
	1959	Check	85	25	29.4	18.1	36	108	25.0	11.1	25.0	7.4
		Infest.	118	50	42.4	11.9	76	164	46.1	55.5	5.3	0.6
P.O.J. 213	1958	Check	78	45	57.6	0.0	24	62	20.8	1.6	29.2	14.5
		Infest.	288	105	36.5	9.2	61	139	68.9	51.1	1.6	2.2
	1959	Check	81	47	58.0	11.8	32	100	12.5	7.0	0.0	0.0
		Infest.	89	35	39.3	13.2	32	119	37.5	32.8	0.0	0.0
P.O.J. 234	1958	Check	106	27	25.4	10.8	11	28	54.4	11.1	0.0	0.0
		Infest.	207	75	36.3	18.9	24	93	45.8	20.4	0.0	3.2
	1959	Check	91	40	43.9	13.5	8	80	12.5	12.5	0.0	0.0
		Infest.	116	39	33.6	20.3	30	130	43.3	16.9	0.0	0.0

Table VIII. Continued.

Sugarcane Variety	Year Examined	Treatment	No. Intern. Planted	Intern. Diseased No.	Intern. Diseased %	% Bud Germin.	No. Isolations Made From Nodes	No. Isolations Made From Intern.	% Cultures Obtained Phytophthora Nodes	% Cultures Obtained Phytophthora Intern.	% Cultures Obtained Physalospora Nodes	% Cultures Obtained Physalospora Intern.
Co. 281	1958	Check	66	26	39.3	10.9	30	84	16.7	0.0	40.0	27.4
		Infest.	130	73	56.2	0.0	46	185	45.7	30.8	19.6	10.8
	1959	Check	58	36	62.1	1.5	58	140	17.2	14.3	5.2	4.3
		Infest.	91	41	45.1	5.0	78	183	15.4	12.0	0.0	0.0
Co. 290	1958	Check	88	31	35.2	34.3	25	95	32.0	21.1	28.0	23.2
		Infest.	144	89	61.8	18.4	45	148	66.7	50.7	6.7	20.3
	1959	Check	89	43	48.3	17.0	36	80	16.7	16.3	13.9	1.3
		Infest.	124	44	35.5	11.3	83	216	32.5	33.3	3.6	2.3
N.Co. 310	1958	Check	73	14	19.1	36.4	8	72	0.0	0.0	12.5	2.8
		Infest.	126	36	28.6	37.7	18	123	55.6	28.4	0.0	2.4
	1959	Check	86	31	36.0	23.9	34	98	23.5	14.3	3.2	1.0
		Infest.	130	40	30.8	22.0	30	110	20.0	27.3	3.3	3.6
C.P. 807	1958	Check	75	15	20.0	23.2	0	48	0.0	0.0	0.0	6.3
		Infest.	170	45	26.5	24.0	23	64	43.5	7.8	13.0	7.8
	1959	Check	109	22	20.2	12.7	34	77	0.0	3.9	17.6	0.0
		Infest.	139	29	20.8	13.1	40	106	40.0	25.5	27.5	0.0

Table VIII. Continued.

Sugarcane Variety	Year Examined	Treatment	No. Intern. Planted	Intern. Diseased No.	Intern. Diseased %	% Bud Germin.	No. Isolations Made From Nodes	No. Isolations Made From Intern.	% Cultures Obtained Phytophthora Nodes	% Cultures Obtained Phytophthora Intern.	% Cultures Obtained Physalospora Nodes	% Cultures Obtained Physalospora Intern.
C.P.28-19	1958	Check	58	29	50.0	2.5	29	76	10.3	15.8	44.8	26.3
		Infest.	98	48	49.0	4.2	22	86	77.3	44.2	13.6	17.4
	1959	Check	76	11	14.5	8.6	20	76	10.0	6.6	0.0	0.0
		Infest.	104	16	15.4	11.5	68	159	17.6	24.5	7.4	0.0
C.P.29-116	1958	Check	81	23	28.4	22.4	10	49	20.0	0.0	0.0	19.8
		Infest.	131	71	54.1	21.6	37	115	32.4	39.1	19.2	6.9
	1959	Check	84	31	36.9	12.5	20	85	20.0	10.6	5.0	14.1
		Infest.	122	71	58.1	9.1	48	126	41.7	33.3	4.2	2.4
C.P.29-320	1958	Check	64	37	57.8	4.1	47	120	46.8	28.3	2.1	3.3
		Infest.	114	59	51.7	16.0	35	188	31.4	3.2	22.9	9.1
C.P.34-120	1958	Check	69	32	46.4	14.9	27	37	29.6	24.3	11.1	2.7
		Infest.	112	60	53.5	12.5	59	167	69.5	44.9	8.5	8.4
	1959	Check	90	51	56.7	1.1	50	157	18.0	13.4	4.0	0.6
		Infest.	121	58	47.9	9.4	86	186	52.3	40.9	0.0	0.5

Table VIII. Continued.

Sugarcane Variety	Year Examined	Treatment	No. Intern. Planted	Intern. Diseased No.	Intern. Diseased %	% Bud Germin.	No. Isolations Made From Nodes	No. Isolations Made From Intern.	% Cultures Obtained Phytophthora Nodes	% Cultures Obtained Phytophthora Intern.	% Cultures Obtained Physalospora Nodes	% Cultures Obtained Physalospora Intern.
C.P.36-13	1958	Check	72	32	44.5	33.3	13	84	10.0	6.0	15.4	4.8
		Infest.	121	56	46.2	32.9	24	143	55.0	18.4	4.2	3.4
	1959	Check	90	42	46.7	10.4	34	108	14.7	12.0	8.8	4.6
		Infest.	103	39	37.8	9.5	46	160	43.5	40.6	6.5	1.8
C.P.36-105	1958	Check	95	9	9.5	35.8	0	41	0.0	0.0	0.0	2.4
		Infest.	141	52	36.8	34.5	20	106	30.0	11.3	0.0	1.9
	1959	Check	87	47	54.0	13.3	15	94	53.3	9.6	0.0	0.0
		Infest.	111	39	35.1	8.9	78	120	33.3	28.3	2.6	1.7
C.P.43-47	1958	Check	85	37	43.5	26.7	11	71	0.0	1.4	0.0	0.0
		Infest.	141	78	55.3	29.2	12	160	33.3	33.1	0.0	1.2
	1959	Check	66	52	26.2	23.2	16	112	18.7	8.9	37.5	15.2
		Infest.	106	60	56.6	17.7	37	156	44.7	43.6	0.0	3.8
C.P.44-101	1958	Check	88	23	26.1	35.9	13	73	46.2	10.9	0.0	12.3
		Infest.	143	32	22.4	32.6	20	132	50.0	34.8	0.0	2.3
	1959	Check	85	41	48.2	24.5	26	102	11.5	8.8	15.4	2.0
		Infest.	115	54	47.0	23.3	28	140	53.5	31.4	3.6	0.0

Table VIII. Continued.

Sugarcane Variety	Year Examined	Treatment	No. Intern. Planted	Intern. Diseased No.	Intern. Diseased %	% Bud Germin.	No. Isolations Made From Nodes	No. Isolations Made From Intern.	% Cultures Obtained Phytophthora Nodes	% Cultures Obtained Phytophthora Intern.	% Cultures Obtained Physalospora Nodes	% Cultures Obtained Physalospora Intern.
C.P.44-154	1958	Check	69	16	23.2	48.8	16	70	0.0	0.0	56.2	7.1
		Infest.	110	91	82.7	6.6	60	189	65.0	54.0	8.3	3.7
C.P.44-155	1958	Check	67	28	41.7	44.9	12	56	0.0	0.0	8.3	0.0
		Infest.	119	68	57.1	21.8	44	179	27.3	19.6	0.0	1.1
	1959	Check	88	29	32.9	11.7	6	28	83.3	22.2	0.0	0.0
		Infest	103	31	30.1	12.6	12	27	66.7	33.3	0.0	0.0
C.P.47-193	1958	Check	123	16	13.0	17.5	24	30	41.7	6.7	0.0	0.0
		Infest.	177	45	25.4	23.3	32	74	46.9	31.1	0.0	0.0
	1959	Check	90	26	28.8	23.7	16	79	6.3	1.3	6.3	2.5
		Infest.	125	33	26.4	21.4	36	131	44.4	36.6	0.0	0.0
C.P.48-103	1958	Check	114	52	45.6	35.2	21	73	19.0	10.9	0.0	9.6
		Infest.	180	70	38.8	31.9	33	94	18.5	22.3	9.1	4.3
	1959	Check	92	45	48.9	16.4	18	63	33.3	23.8	0.0	0.0
		Infest.	143	22	15.4	18.9	4	44	25.0	45.5	0.0	0.0

Table VIII. Continued.

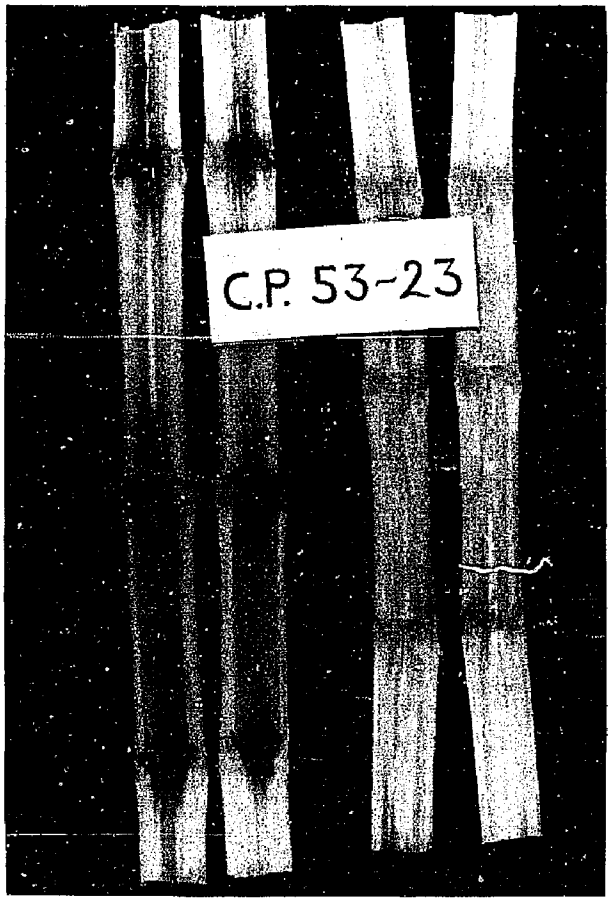
Sugarcane Variety	Year Examined	Treat-ment	No. Intern. Planted	Intern. Diseased No.	Intern. Diseased %	% Bud Germin.	No. Isolations Made From Nodes	No. Isolations Made From Intern.	% Cultures Obtained Phytophthora Nodes	% Cultures Obtained Phytophthora Intern.	% Cultures Obtained Physalospora Nodes	% Cultures Obtained Physalospora Intern.
C.P.51-21	1958	Check	88	37	42.0	40.0	19	104	0.0	18.3	0.0	1.0
		Infest.	149	70	46.9	36.4	25	129	4.0	15.5	4.0	4.6
	1959	Check	75	46	61.3	14.6	24	100	8.3	11.0	8.3	5.0
		Infest.	109	33	32.0	20.0	18	72	33.6	26.3	0.0	8.3
C.P.52-68	1958	Check	78	29	37.1	22.6	42	82	42.9	20.7	0.0	0.0
		Infest.	127	39	30.7	9.4	44	156	81.8	34.6	0.0	0.0
	1959	Check	73	19	26.0	17.5	24	72	37.5	50.0	4.2	4.2
		Infest.	96	28	29.2	16.1	35	105	34.3	42.9	0.0	1.0
C.P.53-1	1958	Check	87	31	35.6	27.9	14	80	0.0	6.3	7.1	12.5
		Infest.	140	75	53.6	22.2	38	176	52.6	36.9	5.3	10.2
	1959	Check	72	57	79.1	5.2	20	90	70.0	27.8	0.0	0.0
		Infest.	92	70	76.1	5.3	48	133	33.3	36.8	4.2	0.0
C.P.53-15	1958	Check	82	19	23.2	12.5	9	44	11.1	4.5	0.0	2.3
		Infest.	110	48	43.6	11.3	59	143	82.9	51.7	1.7	1.4

Table VIII. Continued.

Sugarcane Variety	Year Examined	Treatment	No. Intern. Planted	Intern. Diseased No.	Intern. Diseased %	% Bud Germin.	No. Isolations Made From Nodes	No. Isolations Made From Intern.	% Cultures Obtained Phytophthora Nodes	% Cultures Obtained Phytophthora Intern.	% Cultures Obtained Physalospora Nodes	% Cultures Obtained Physalospora Intern.
C.P.53-22	1958	Check	75	54	72.0	15.1	10	29	40.0	34.5	0.0	0.0
		Infest.	130	84	64.6	10.6	34	94	56.2	59.6	5.9	1.1
	1959	Check	72	54	75.0	11.4	54	135	27.8	14.1	11.1	8.9
		Infest.	88	68	77.2	10.2	108	198	53.7	41.4	11.1	3.0
C.P.53-23	1958	Check	80	41	51.2	23.2	55	110	52.7	28.2	7.3	6.4
		Infest.	139	83	59.7	6.3	85	155	52.0	40.0	6.7	9.7
C.P.55-30	1958	Check	82	24	29.2	20.0	17	68	52.9	42.6	11.7	4.4
		Infest.	121	48	39.7	28.5	15	102	66.7	34.3	0.0	1.0
	1959	Check	81	15	18.5	20.2	46	92	23.9	18.5	0.0	7.6
		Infest.	102	26	25.5	13.5	42	100	59.5	39.0	0.0	0.0
TOTALS:	1958	Check	2121	751	35.4	23.6	494	1707	28.7	12.5	13.8	8.6
		Infest.	3863	1700	44.0	19.5	963	3495	52.9	33.6	6.4	4.6
	1959	Check	1820	810	43.8	14.4	627	2075	21.4	13.7	8.0	3.9
		Infest.	2447	926	37.8	14.5	1054	2885	36.1	34.7	4.5	1.3

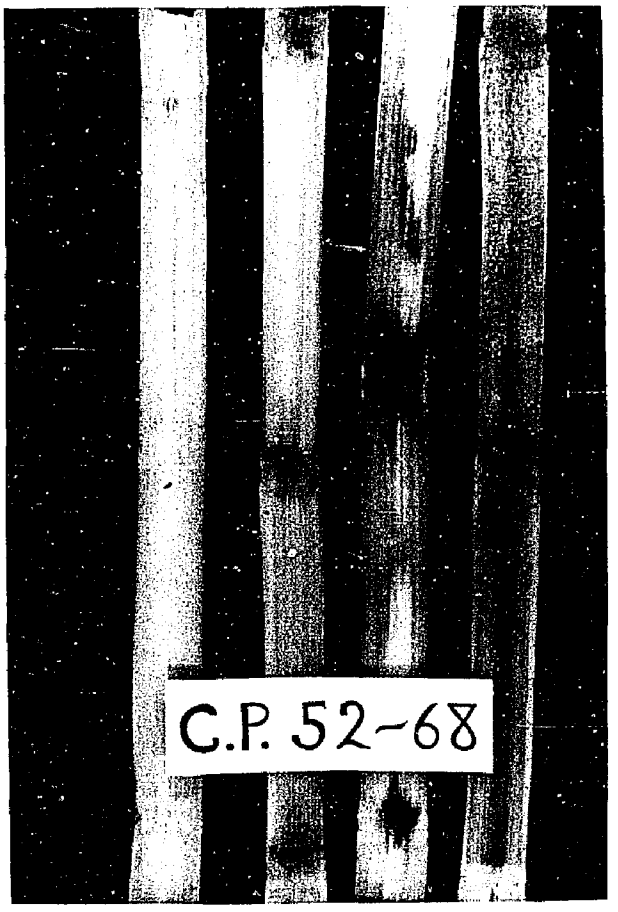
Varieties C.P. 29-320, C.P. 44-154, C.P. 53-15 and C.P. 53-23 were not repeated in 1959.

PLATE XV



Early nodal infection and spread of Phytophthora in seed pieces of the variety C.P. 53-23. Check at right.

PLATE XVI



Progressive spread of Phytophthora from nodal regions into the internodal tissue in seed pieces of the variety C.P. 52-68. Check at left.

isolations made from diseased tissue of both nodes and internodes. These data strongly indicate differences in varietal susceptibility to the pathogen.

From the three monthly examinations made both in the greenhouse and in the laboratory, by observing the condition of the seed pieces, including the amount of rotting in the internodes, and the number of Phytophthora cultures isolated from the diseased nodes and internodes, the varieties tested are divided in regard to their disease resistance as follows:

Resistant:	Intermediate:	Susceptible:
C.P. 29-116	P.O.J. 234	P.O.J. 36
C.P. 29-320	Co. 281	P.O.J. 213
C.P. 36-105	N.Co. 310	Co. 290
C.P. 43-47	C.P. 807	C.P. 28-19
C.P. 44-155	C.P. 44-101	C.P. 34-120
C.P. 48-103	C.P. 47-193	C.P. 36-13
C.P. 51-21	C.P. 53-1	C.P. 44-154
	C.P. 53-23	C.P. 52-68
		C.P. 53-15
		C.P. 53-22
		C.P. 55-30

The variety found to be the most susceptible to the seed piece rot was C.P. 44-154 (Plate I). It will have to be emphasized again, however, that this rating is strictly based on the testing of these varieties under weather conditions extremely favorable for the development of Phytophthora seed piece rot, as was experienced during the winter and spring of 1957-1958.

Fortunately, the winter of 1958 was much less favorable for disease

development, since the temperature during the 3 months after planting was considerably higher as was recorded for 1957, and the average amount of rainfall was not over 1.4 inches as compared to 4.4 inches for 1958. It can be seen from Table VIII, that the percentage diseased internodes and the percentage of Phytophthora cultures obtained from isolations of diseased nodes and internodes, is considerably lower for the year 1959 as compared with the previous year. The low percentage bud germination observed during that year is presumed to be due to unfavorably dry weather conditions, which were not conducive for good seed piece germination. In comparing the overall totals and averages of all the varieties combined, the same conclusions can be drawn as have been mentioned before.

When the first stand count was made on April 15, 1959, of the varieties planted in the fall of 1958, it was observed that in the infested soil several varieties had only a very few shoots above the ground as compared to their checks. When the other stand counts were made, at monthly intervals from the first one, it was found that those varieties kept failing to germinate, while the limited stand also had much less plant height than its check. The data of these counts are presented in Table IX.

Even though the weather conditions during the winter of 1958-1959 were much less favorable for Phytophthora seed piece rot, several varieties mentioned as being susceptible under the 1957-1958 conditions,

Table IX. Total number of plants and average plant height of many past and present commercial sugarcane varieties and some unreleased experimental test field varieties, planted in soil artificially infested with P. megasperma under field conditions, recorded at monthly intervals during the spring of 1959.

Sugarcane Variety	Treatment	No. Stalks Planted	Number of Plants				No. Heavy Stalks	Height of Plants			
			4/15	5/15	6/15	7/15		4/15	5/15	6/15	7/15
P.O.J. 36	Check	9	1	3	8	13	2	10	17	20	38
	Infest.	12	--	--	--	--	--	--	--	--	--
P.O.J. 213	Check	9	6	8	14	16	6	13	21	24	51
	Infest.	12	--	--	--	--	--	--	--	--	--
P.O.J. 234	Check	9	5	12	29	34	15	10	19	34	58
	Infest.	12	--	--	--	--	--	--	--	--	--
Co. 281	Check	9	0	4	14	14	5	0	17	29	50
	Infest.	12	--	--	--	--	--	--	--	--	--
Co. 290	Check	9	6	10	29	32	14	14	19	40	60
	Infest	12	--	--	--	--	--	--	--	--	--
N.Co. 310	Check	9	7	13	35	25	15	13	23	44	64
	Infest.	12	8	24	60	49	26	8	21	40	64
C.P. 807	Check	9	3	11	25	21	7	9	12	35	51
	Infest.	12	10	24	44	44	27	8	20	47	62
C.P. 28-19	Check	9	4	10	25	25	18	12	24	49	58
	Infest.	12	3	5	8	7	4	8	19	41	61

Table IX. Continued.

Sugarcane Variety	Treatment	No. Stalks Planted	Number of Plants				No. Heavy Stalks	Height of Plants			
			4/15	5/15	6/15	7/15		4/15	5/15	6/15	7/15
C.P. 29-116	Check	9	6	8	20	22	6	12	16	34	55
	Infest.	12	1	7	12	14	7	14	21	37	63
C.P. 34-120	Check	9	2	4	15	19	6	13	24	34	58
	Infest.	12	2	3	9	9	5	10	24	45	62
C.P. 36-13	Check	9	3	8	28	30	17	14	20	37	61
	Infest.	12	0	1	1	2	0	0	14	26	35
C.P. 36-105	Check	9	5	11	33	36	18	11	24	50	66
	Infest.	12	0	2	6	12	4	0	19	32	53
C.P. 43-47	Check	9	8	12	31	30	13	12	23	45	61
	Infest.	12	12	35	60	65	40	14	19	46	72
C.P. 44-101	Check	9	3	8	18	28	14	11	21	47	68
	Infest.	12	10	24	66	66	42	16	22	56	80
C.P. 44-155	Check	9	5	14	55	42	23	11	26	50	70
	Infest.	12	1	10	25	31	15	13	18	35	65
C.P. 47-193	Check	9	6	13	39	49	22	14	24	48	68
	Infest.	12	2	17	66	72	40	13	23	38	71

Table IX. Continued.

Sugarcane Variety	Treatment	No. Stalks Planted	Number of Plants				No. Heavy Stalks	Height of Plants			
			4/15	5/15	6/15	7/15		4/15	5/15	6/15	7/15
C.P. 48-103	Check	9	10	14	34	39	19	17	23	44	63
	Infest.	12	15	24	78	75	42	15	22	40	70
C.P. 51-21	Check	9	1	8	31	32	16	11	19	35	61
	Infest.	12	2	9	26	27	19	10	15	36	59
C.P. 52-68	Check	9	9	20	34	35	22	11	22	45	71
	Infest.	12	5	8	28	27	13	13	18	35	58
C.P. 53-1	Check	9	3	10	28	28	15	11	21	48	68
	Infest.	12	1	2	9	11	8	9	23	28	50
C.P. 53-22	Check	9	7	9	41	36	17	12	23	37	64
	Infest.	12	2	6	17	20	12	10	24	33	54
C.P. 55-30	Check	9	2	10	28	28	18	11	20	45	63
	Infest.	12	0	1	4	6	1	0	14	28	50

No records were obtained of the varieties P.O.J. 36, P.O.J. 213, P.O.J. 234, Co. 281 and Co. 290 planted in artificially infested soil.

were still found to be susceptible in the latter field test. Based on the 1958-1959 seed piece examination test and the separate stand count made of the same varieties during the spring of 1959, the varietal reaction rating is set as follows:

Resistant:	Intermediate:	Susceptible:
N.Co. 310	C.P. 807	C.P. 28-19
C.P. 29-116	C.P. 36-105	C.P. 34-120
C.P. 43-47	C.P. 44-155	C.P. 36-13
C.P. 44-101	C.P. 52-68	C.P. 53-1
C.P. 47-193		C.P. 53-22
C.P. 48-103		C.P. 55-30
C.P. 51-21		

This listing excludes the 4 varieties not repeated for 1959 and also the P.O.J. and Co. varieties, since no stand count could be obtained of these. On the basis of the seed piece examination test of 1957-1958, however, the varieties C.P. 44-154 and C.P. 53-15 were found to be very susceptible during that year, while on the basis of the 1958-1959 test, the P.O.J. and Co. varieties are suggested for listing under the intermediate column, with the exception of Co. 281, which variety will move to the resistant side.

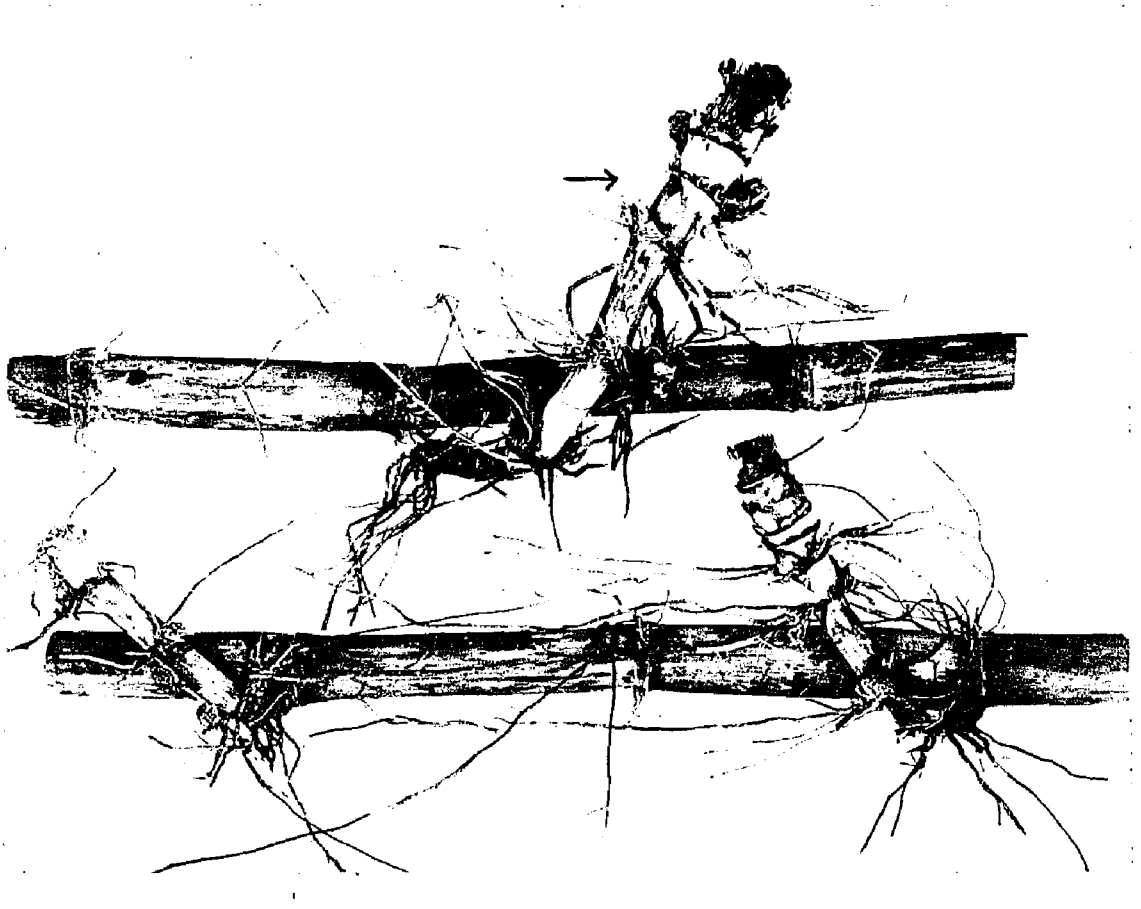
Survey of the Presence of Phytophthora Seed Piece Rot in Louisiana During the Spring of 1958

Since the winter of 1957 was very wet, while temperatures were rather low for several months, it was thought to be of some importance to check the Louisiana sugarcane belt for the presence of Phytophthora rot. A limited survey was made during March and April of 1958 and seed pieces were dug from gaps in the rows, in which the plant cane had apparently failed to germinate. Since C.P. 44-101 is the most dominant commercial variety in the state today, seed pieces of this variety were collected more than of any other variety. At the time the collecting was done, a note was made of the date the seed cane had been planted. Isolations were made from all diseased internodes, and the percentage cultures of Phytophthora and Physalospora were recorded. The data are presented in Table X.

It can be seen from this table, that in many cases the higher percentages of Phytophthora cultures were obtained from seed cane planted in late September, October or November, while cane planted in August or early September did not reveal as much Phytophthora. Other factors, however, like temperature, amount of rainfall and soil type for the specific locations are not included, which play also an important role in the occurrence of Phytophthora rot.

Several seed pieces of the variety C.P. 36-13 were brought into the laboratory, which had been planted in August of 1957. These canes

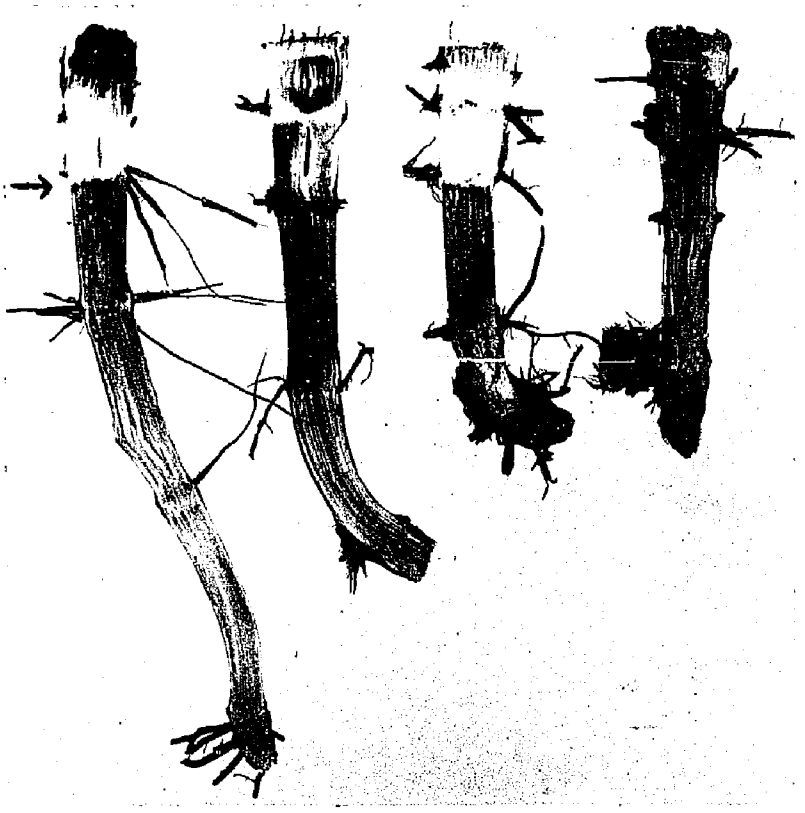
PLATE XVII



Young stubble produced from summer planted seed cane of the variety C.P. 36-13, diseased with Phytophthora rot.

⟶ indicates soil line.

PLATE XVIII



Typical watersoaked appearance of split young stubble from summer planted seed cane of the variety C.P. 36-13, diseased with Phytophthora rot.

⟶ indicates soil line.

had germinated during the early fall of that year producing a small underground "rhizome," which in turn was killed back above the ground, after freezing temperatures had set in (Plate XVII). The original seed piece or mother stalk was brown in color and was almost completely deteriorated.

Upon splitting and examining this young stubble, it was found that the whole "rhizome" was watersoaked in appearance and in most cases had an additional purple discoloration, combined with the typical fermenting odor of Phytophthora rot (Plate XVIII). In both of these plates a definite line (see arrow) can be noticed between the diseased underground part of the stubble and the yet healthy portion above ground. P. megasperma was consistently re-isolated from all the diseased stubbles collected, indicating that Phytophthora rot can also cause some damage to seed cane planted in the summer, by rotting and killing the young stubble, if weather conditions are at an optimum for development of the disease.

Table X. Survey of the presence of Phytophthora rot in plant cane of several sugarcane varieties collected from different locations of the sugarcane belt in Louisiana during the spring of 1958.

Plantation	Location	Sugarcane Variety	Time Planted	Time Examined	No. Isolations Made From Internodes	% Cultures Obtained	
						Phytophthora	Physalospora
Evanhall	Donaldsonville	C.P.44-101	Aug.'57	March'58	78	0.0	18.0
Catherine	Bayou Goula	C.P.44-101	Nov.'57	March'58	105	39.1	1.0
Columbia	Edgard	C.P.48-103	Sept.'57	March'58	15	0.0	0.0
		C.P.44-101	Aug.'57	March'58	64	0.0	20.3
		C.P.47-193	Aug.'57	March'58	34	0.0	14.7
		C.P.43-47	Aug.'57	March'58	130	11.5	19.2
St. James Coop.	St. James	C.P.44-101	Sept.'57	March'58	164	4.3	11.0
Glynn's Farm	New Roads	C.P.44-101	Sept.'57	March'58	16	50.0	0.0
Halfway	Bellrose	C.P.44-101	Aug.'57	April'58	67	16.4	0.0
John Tregre	Napoleonville	C.P.44-101	Oct.'57	April'58	166	12.7	12.7
		C.P.44-101	Oct.'57	April'58	66	6.1	16.7

Table X. Continued.

Plantation	Location	Sugarcane Variety	Time Planted	Time Examined	No. Isolations Made From Internodes	% Cultures Obtained Phytophthora	% Cultures Obtained Physalospora
Shell Hill	Vacherie	C.P.44-101	Sept.'57	April'58	77	40.3	26.0
Burley's Farm	Youngsville	C.P.44-101	Oct.'57	April'58	41	0.0	9.8
		N.Co. 310	Oct.'57	April'58	97	23.7	1.0
Sterling Sugars	Franklin	C.P.44-101	Aug.'57	April'58	33	9.4	0.0
Youngs Indust.	Youngsville	Co. 290	Aug.'57	April'58	105	9.5	3.8
Billeaud Sugars	Broussard	C.P.44-101	Aug.'57	April'58	34	0.0	2.6
LSU Exp. Sta.	Baton Rouge	C.P.34-120	Nov.'57	April'58	45	26.7	2.2
		C.P.29-320	Nov.'57	April'58	69	33.3	7.2

DISCUSSION

Many plant diseases are known to be caused by more than one organism, and also in the group of diseases caused by members of the genus Phytophthora there are several, which are caused by two or more species of the genus. Probably the earliest report came from Ireland in 1913, where Pethybridge (63) described the pink rot disease of potatoes and identified the causal organism as a new species of Phytophthora, which he named P. erythroseptica. According to Butler and Jones (15), P. cryptogea, P. cactorum and P. megasperma may also produce, to a greater or lesser extent, the symptoms of pink rot.

In the same year, Pethybridge and Lafferty (64) described a disease of tomato and created another new species, P. cryptogea. The disease consists of a damping-off and a foot rot phase and both phases are now known to be caused by two species of Phytophthora, namely P. cryptogea and P. parasitica. Another Phytophthora disease of tomato was described by Sherbakoff (73) from Florida, which he named buckeye rot. He also described a new species and named it P. terrestria, which was later designated as a synonym of P. parasitica (85). Tompkins and Tucker (83), however, found the same disease in California to be incited by P. capsici and P. drechsleri, and later, Ramakrishnan and Soumini (66), in India, attributed the disease to P. palmivora. In the ornamental

diseases, shanking of tulip is caused by two species of Phytophthora, namely P. cryptogea and P. erythroseptica.

Phytophthora seed piece rot of sugarcane can now also be added to the list of Phytophthora diseases caused by more than one species of Phytophthora. For many years, one of the species has been known to be P. erythroseptica, while the other was never identified due to the complete lack of fruiting structures of the isolate on common culture media. It was referred to as the "sterile" Phytophthora isolate.

It has been a known fact for quite some time that certain species of Phytophthora do not produce sporangia and/or oospores under all conditions or on all culture media. Many investigators have tried numerous materials to induce fruiting, like the use of different hosts, fruit, soil leachate, sucrose solutions and others (7, 8, 43, 51, 52, 59). Also the use of pairing certain cultures of Phytophthora has often resulted in the production of sexual organs (5, 22, 77).

Since most of these attempts were already used by two previous investigators of sugarcane seed piece rot (71, 75), other techniques were tried during this study to induce the "sterile" isolate to produce fruiting structures. Since sterilized oat grains were used in the present investigation for soil infestation in different experiments it was felt that the fungus may fruit upon this medium. Oats thickly covered with the fungus, were examined by taking scrapings of the surface of the grains and upon microscopic examination, numerous oospores were observed.

Induction of fruiting structures in Phytophthora, by using whole oats as the culture medium has been known for many years. Since the time that the potato blight fungus started causing considerable losses to the European potato industry (1845), until the early part of the twentieth century, nobody had been able to find the sexual spores of P. infestans. It was not until 1910, that Clinton (19) made the long awaited discovery, by using oats or oat juice in different culture media. He found that oogonia were produced quite frequently and that, only with the addition of oat juice, the antheridia would fertilize the oospheres. Clinton's interesting theory was, as expressed by Large (50), that "it required an old man's aphrodisiac - a little Spanish Fly - to revive its male vigour." The oat juice contained a phosphorized fat substance, called lecithin, which was known to be a constituent of nerve tissue and spermatozoa. Recently, Zentmyer (99) reported from California, that a chemical substance found in feeder roots of the Mexican avocado, stimulated sexual reproduction in P. cinnamomi. He could not, however, induce fruiting in P. capsisci, P. cambivora, P. citrophthora, P. cryptogea, P. palmivora and P. parasitica with this material.

After the typical characteristics of the sugarcane isolate were recorded, a review was made of all the known Phytophthora species, in order to compare these characteristics and also the measurements of the different fruiting structures. The four selected Phytophthora species, which most closely resembled the sugarcane isolate morphologically,

were P. cinnamomi, P. drechsleri, P. cryptogea and P. megasperma. Also P. erythroseptica was used for comparison, since this species had already been described from sugarcane seed pieces.

It was decided, after close comparative studies, that because of non-papillate sporangia produced on long sporangiophores with excessive proliferation, combined with the formation of typical intercalary chlamydospores, paragynous antheridia and oospores with thick endospores, the Phytophthora isolate from sugarcane is identified as Phytophthora megasperma Drechsler. This identification has been confirmed by Dr. Barrett at Berkeley, California and Miss Waterhouse at Kew, England, who are two of the world's authorities on the genus Phytophthora.

In order to obtain additional proof of this identification, more comparative studies were undertaken in respect to pathogenicity and growth-temperature relations of the different isolates. When cultures of P. megasperma and the sugarcane Phytophthora isolate were inoculated into eggplant, apples, and oranges, they reacted similarly in respect to their pathogenicity, which results also confirm those found by Gooi et al. (39) and Wager (89), who inoculated similar fruit with cultures of P. megasperma, isolated from peach trees and citrus fruit respectively. Upon inoculation of seed pieces of the varieties Co. 290 and C.P. 34-120, the two isolates reacted again similarly, while both were found to be more pathogenic than the type culture of P. erythroseptica. This confirms in turn the findings by previous investigators of sugarcane seed piece rot

(71, 75, 80), who stated that the "sterile" Phytophthora isolate was considerably more pathogenic than P. erythroseptica described from sugarcane.

It was found in comparative temperature experiments, that on potato dextrose agar, the sugarcane isolate grew closely parallel to P. megasperma at the complete temperature range from 5° to 35° C., as contrasted with the other type cultures tested. This was also found to be true on 5 additional culture media tested. When all the isolates were grown at a temperature of 5° C. and were examined after one month incubation, it was found that only P. megasperma and the sugarcane Phytophthora isolate had grown a considerable amount (50 mm colony diameter) in plate cultures. This seems a very interesting observation, since it indicates (1) once again the close identity of the sugarcane isolate to P. megasperma and (2) that it seems this species is apparently well capable of survival and growth at this low temperature and at the range of 5°-20° C. Since the fungus is able to grow at these low temperatures, it may serve to explain why Phytophthora seed piece rot is most severe during winters of low temperatures and high rainfall.

During the two years in which the present investigations were undertaken, the winter and spring of 1957-1958 was very wet, accompanied by low temperatures, while during 1958-1959, however, the winter and spring were mild, with below normal rainfall. Under these two conditions, many sugarcane varieties, including past and present commercial

varieties and some unreleased test field varieties, were tested under field conditions. It was evident that the year 1957-1958 was much more favorable for development of Phytophthora seed piece rot than the following year. This was proven by the number of Phytophthora cultures obtained from the diseased nodes and internodes of the seed pieces. It was observed in the spring of 1958, that during the successive examinations of the seed pieces at monthly intervals, the amount of Phytophthora infection increased from month to month and also the spread of the fungus inside the canes, which was revealed by the increasing numbers of Phytophthora cultures isolated from the seed pieces.

Also the date of planting seed cane can be of importance with respect to Phytophthora rot. In the limited survey conducted during the spring of 1958, it was found that seed cane planted from late September until November gave higher percentages of Phytophthora cultures from diseased internodes than that planted in August or early September. In a recent publication, Hebert and Matherne (40) pointed out other advantages and disadvantages of the date of planting seed pieces in respect to yields of cane and sugar. They also stated that the depth of placement of the seed pieces with respect to the water furrows, should be varied from relatively deep in the better-drained, light soils to relatively shallow placement in the heavier, poorly-drained soils. These facts are of importance indirectly in respect to Phytophthora seed piece rot.

In the present study it was also found in some cases, that the young stubble produced by seed cane of the variety C.P. 36-13, planted in August of 1957, was watersoaked in appearance and had completely deteriorated. Since Phytophthora was consistently isolated from the young "false stubbles," of August planted cane, this seems to indicate that Phytophthora rot may be of importance in the stand failures which occur following a winter of low temperatures and high rainfall. This had in the past already been attributed to red rot by Edgerton and his associates (31, 33), while recently, Singh (74) reported that from isolations made of shoots from these young stubbles, produced by August planted seed cane, 24.3 per cent of the underground buds were infected by the red rot fungus.

In respect to the failures of P.O.J. 213 and Co. 290 during the early 1930's and 1940's respectively, it was suggested by previous investigators that both varieties went down most likely with red rot (3, 36). In comparing the weather conditions of these two historical periods, it was found that the fall of 1929 was one of the wettest falls on record (October through January, 41.2 inches), while during the following winter the number of hours below freezing (November - February, 224 hours) was higher than for previous or later winters (31). During the fall of 1939, the temperature ranged from 70.6° F. in October to 56.6° F. in December, while the average amount of rainfall was 3.0 inches. The spring of 1940 had a temperature range from January until March of

41.2°-61.4° F. and an average precipitation of 4.7 inches.

In the present field testing program during the cold and wet year 1957-1958, the varieties P.O.J. 213 and Co. 290 were found to be quite susceptible to Phytophthora, while during the following dry and mild year, they were much less susceptible to the pathogen. Since the years 1929-1930 and to a lesser extent 1939-1940 were both rather cold and very wet, and because of the fact that relatively high numbers of Phytophthora cultures were recovered from both these varieties during the spring of 1958, it is suggested that Phytophthora seed piece rot might have played an important additional role in the stand failures of the varieties P.O.J. 213 and Co. 290 in previous years.

In regard to the disease epidemics in the variety C.P. 36-13 in certain areas of the sugarcane belt in the spring of 1947 and 1948, which resulted in the first announcement of Phytophthora seed piece rot and the identification of one of the Phytophthora isolates as P. erythroseptica, it is assumed from the present investigation that this failure was due entirely to that seed piece rot. The average temperature for the months October through December of 1946 ranged from 70.6°-57.2° F., accompanied by 3.8 inches of rainfall during that period of time. The very low average temperature during the winter of 1947, again accompanied by excessive amounts of rainfall, undoubtedly influenced the severity of the disease. Average temperatures were near a record low for both February and March of 1948. These weather data, together with the high number

of Phytophthora cultures and few red rot cultures isolated in this study from seed pieces during the spring of 1958, indicate that Phytophthora rot was entirely responsible for the epidemic in this variety during those two years.

An interesting observation made in the present investigation was the fact that the seed pieces became infected more easily through the nodal regions than through the cut ends of the canes, as would ordinarily be expected, and which had been found to be the case in pineapple disease and black rot. The occurrence of this nodal infection at the yet ungerminated root primordia and buds, is therefore an important factor in the development of Phytophthora seed piece rot. When cane is planted in the fall of the year and cold weather sets in early, accompanied by heavy precipitation which will keep the soil saturated, Phytophthora seed piece rot can become of much importance, especially to the more susceptible varieties. It is felt that also a cool wet spring is important with respect to inducement of Phytophthora rot. A cool spring prevents the cane from germinating, but not the disease from occurring.

SUMMARY

In the present investigation, a study was made of the Phytophthora seed piece rot of sugarcane in Louisiana and of the causal organism of the disease. Since one of the species of Phytophthora had previously been identified as P. erythroseptica Pethyb., while the other more pathogenic isolate had never been described and identified, an attempt was made in this study to identify that virulent species.

Upon growing the unknown isolate on sterilized oat grains and later placing these grains in water, many sporangia and oospores were found to be produced by the fungus. A complete description of this isolate has been given including all measurements and other characteristics of the fruiting structures. After comparing all the morphological characteristics of the Phytophthora isolate with those of several type cultures of other Phytophthora species, and after making additional comparative tests of these cultures in respect to growth-temperature relations and pathogenicity, the virulent Phytophthora isolate from sugarcane is identified as P. megasperma Drechs. This identification has been confirmed by two authorities on the genus Phytophthora.

In order to study the basic method of infection of seed pieces by Phytophthora, an experiment was set up in which 2-eyed seed pieces of the varieties N.Co. 310 and C.P. 36-13 were planted in sterilized soil, artificially infested with P. megasperma. These seed pieces were dug

and examined at 2, 3, 5, 7 and 9 weeks after planting, and it was found evident in the first examination that many of the root primordia and buds were red discolored, swollen and infected with Phytophthora. At the end of 9 weeks, N.Co. 310 had 55.5 per cent of its root primordia and 70.0 per cent of its buds infected, while for C.P. 36-13, the percentages were 67.9 and 80.0 respectively. It was also observed in this experiment, that none of the root primordia and buds, of seed pieces in infested soil which had escaped infection by the pathogen, had germinated, while the seed pieces in the non-infested soil had 70.0-80.0 per cent bud germination and an abundant root system at every node. From additional preliminary laboratory studies, it is suggested that a toxin is possibly produced by the fungus, which inhibits the germination process.

In order to study the rate of spread of Phytophthora inside the cane tissue, 2-eyed seed pieces of the same two varieties were inoculated with P. megasperma. The seed pieces were planted in sterilized soil and were dug for examination at 10, 15, 20, 30 and 50 days after inoculation. Upon examination of the canes, the typical pink discoloration and watersoaked appearance of the tissue was observed, which later turned purplish in color, giving off an ether-like odor. The diseased portion of the internode reached the nodal regions 20 days after inoculation, while after 50 days the internode had almost entirely deteriorated. Phytophthora spread more rapidly in the variety N.Co. 310 than in C.P. 36-13.

In a comparative susceptibility experiment, the varieties P.O.J. 213, Co. 290 and C.P. 36-13 were tested in regard to red rot and Phytophthora rot. It was found, as had been reported before, that Co. 290 was the most susceptible variety to red rot, while C.P. 36-13 reacted worst in respect to Phytophthora rot. P.O.J. 213 was intermediate between the two varieties. The percentage bud germination of all varieties was observed to be the lowest when canes were inoculated with P. tucumanensis and planted in soil artificially infested with Phytophthora. Non-inoculated seed pieces planted in infested soil had a higher percentage bud germination than those only inoculated with the red rot fungus, except for the variety C.P. 36-13, which in turn also gave the highest percentage of Phytophthora cultures from isolations of diseased nodes and internodes.

An extensive varietal field test was carried out during the winter and spring seasons of 1957-1958 and 1958-1959, in which many past and present commercial sugarcane varieties and some unreleased test field varieties were tested under field conditions in regard to their susceptibility to Phytophthora seed piece rot. The seed pieces were dug and examined at monthly intervals during each spring season, in order to obtain a percentage of diseased internodes and of bud germination. Isolations were also made of all diseased nodes and internodes which had not been deteriorated too far. Two separate plantings of the varieties were made in the fall of 1958, one of which was used to make a stand count

during the following spring.

From the data collected during this two year testing program, it may be concluded that the varieties C.P. 28-19, C.P. 34-120, C.P. 36-13, C.P. 44-154, C.P. 53-1, C.P. 53-15, C.P. 53-22 and C.P. 55-30 are quite susceptible to Phytophthora seed piece rot, while the following varieties were considered resistant: Co. 281, N.Co. 310, C.P. 29-116, C.P. 43-47, C.P. 44-101, C.P. 47-193, C.P. 48-103 and C.P. 51-21.

During the spring of 1958 a survey was made in the Louisiana sugar-cane belt to check the presence of Phytophthora rot in the commercially grown varieties. It was observed that higher percentages of Phytophthora were obtained from seed pieces planted from late September until November than from those planted in August or early September.

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AUTOBIOGRAPHY

Tom van der Zwet was born on April 7, 1932 in Balik Papan, Borneo, Indonesia. He graduated from the Hogere Burger School B at Haarlem, The Netherlands in 1949. In the fall of that year he entered the State College for Tropical Agricultura at Deventer, The Netherlands and received the final certificate in July 1952.

From September 1952 until July 1953 he worked as a "special student" at the Inter American Institute of Agricultural Sciences at Turrialba, Costa Rica, conducting several experiments on the vegetative propagation of coffee.

In September 1953 he entered the Louisiana State University as a Junior and received the Bachelor of Science degree in Botany in May 1955. That same summer he started graduate work in the Department of Botany, Bacteriology and Plant Pathology and obtained the Master of Science degree in Plant Pathology in June 1957. Graduate studies were continued in the fall of that year with specialization in sugarcane pathology.

During the summer of 1958 he was employed by Dugas Pest Control, Inc. to assist the consultant program in connection with the control of the sugarcane borer in Louisiana.

He is now a candidate for the degree of Doctor of Philosophy in Plant Pathology at this University.

PUBLICATIONS

1. van der Zwet, T. 1958. The effect of flooding upon the severity of Pythium root rot. (Abstr.) Phytopath. 48(6): 345-346.

2. _____. 1959. Studies on a Phytophthora isolate causing seed piece rot of sugarcane in Louisiana. (Abstr.) Phytopath. 49(5): 320.

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EXAMINATION AND THESIS REPORT

Candidate: Tom van der Zwet

Major Field: Plant Pathology

Title of Thesis: Studies on Phytophthora Seed Piece Rot of Sugarcane and the Principal Causal Organism P. megasperma Drechs.

Approved:

J. L. Forbes
Major Professor and Chairman

George H. Mickey
Dean of the Graduate School

EXAMINING COMMITTEE:

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Date of Examination:

July 27, 1959